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ANALYSIS OF ORGANIC COMPOUNDS IN TWO KRAFT MILL WASTEWATERS

Lawrence H. Keith

National Environmental Research Center
Athens, Georgia

June 1975

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By

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National Environmental Research Center
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Program Element 1BA027
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ABSTRACT

Wastewaters from two kraft paper mills in Georgia were sampled at various points in the waste treatment systems. Gas chromatography of the organic extracts and identification of many of the specific chemical components by gas chromatography-mass spectrometry provided a "chemical profile" of the effluents. The mills, in different geographical locations, have very similar raw wastewater compositions but different wastewater treatments. In spite of these differences, the treated effluents are qualitatively similar in composition although the quantities of the various components differ. After two years the raw and treated effluents of both mills were re-sampled. Analyses showed that although concentrations of the organics varied, the same compounds are still present. This report was submitted in fulfillment of ROAP 07ABL, Tasks 02 and 03 by SERL, Athens, Georgia. Work was completed as of April 1974.

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SECTION I

CONCLUSIONS

Computer-assisted GC-MS is the best method of identifying unknown volatile organics in wastewaters.

It is now possible to obtain routinely by GC analysis much pertinent data concerning the quantities of specific chemicals being discharged into environmental waters from kraft paper mills.

The efficiency of various types and combinations of existing treatment facilities can now be measured and compared as to their removal of undesirable compounds from kraft paper mill wastewaters.

In mill laboratories, computer-assisted GC analysis can save much time and expense over manual GC.

The GC-MS techniques and extraction/concentration methods employed permitted identification of some compounds in less than 1 part per billion in the wastewaters.

A number of general conclusions can be drawn from this study:

- Raw effluents from kraft pulp mills producing paper from the same raw materials contain essentially the same volatile organics.
- The volatile organic composition of kraft wastewaters remains relatively constant over the years as long as the raw materials remain the same and the process is not changed.
- When peaks in the GC of a pulp mill wastewater have been identified, routine GC analyses on a mill laboratory basis are feasible.
- The relative quantities of the volatile organics in pulp mill wastewaters can fluctuate rapidly.
- Because of rapid fluctuations in the quantities of organics in the wastewaters, composite samples are needed for representative quantitative results.

The wastewaters of the two mills were similar in several respects:

- The raw effluents were very similar qualitatively with regard to volatile organic chemical content. This was expected since both mills produce a kraft linerboard by the same process and from the same raw materials.
- Significant BOD reductions occurred in both treatment systems.
- Total chromatographable organics were significantly reduced by both treatment systems.
- Both treated effluents showed large reductions in resin acid, phenolic, and neutral volatiles content.

Only one point of difference was noted. Total fatty acid content was decreased in Interstate's treated effluent but not in that of Mill "A". Fatty acids in the oxidation of Mill "A" may be biological metabolites from aquatic life in these ponds.

Conclusions can also be reached from a comparison of the effects of biological treatment (Mill "A's" trickling filter and aerated lagoon) versus chemical/biological treatment (Interstate's lime flocculation/stabilization lagoon) on the volatile organic compounds in these wastewaters. All values used in these comparisons came only from the January 1974 composite samples.

- BOD reductions were in the range of 85-90% with both treatments.
- TOC reductions were in the range of 80-85% with chemical/ biological treatment and 60-65% with only biological treatment.
- Total volatile acids and phenols were reduced by 75-80% in both treatments.
- Total resin acids were reduced in concentration by 87% with biological treatment but were still present at about 1.4 mg/liter in the treated effluent. Total resin acids were reduced in concentration by 74% with chemical/biological treatment but were still present at about 6.4 mg/liter in the treated effluent.

- Total phenols were reduced in concentration by 73% with biological treatment and were present at 0.6 mg/liter in the treated effluent. They were reduced by 94% with chemical/ biological treatment and were present at 0.2 mg/liter in the treated effluent.

- Total fatty acids increased 17% in concentration with biological treatment and were present at 1.6 mg/liter in the treated effluent. They decreased by 86% with chemical/biological treatment and were present at 0.2 mg/liter in the treated effluent.

- Biologically treated wastewaters from both mills contained a greater number of fatty acids than did the raw wastewaters. The new fatty acids were branched and oddnumbered carbon compounds.

- Total volatile materials ranged from 1-10% of the TOC.

- Terpenes were reduced by 90% or more by both treatments.

SECTION II

RECOMMENDATIONS

We recommend that further studies by Federal and university laboratories, and especially laboratories associated with the paper mills or the National Council for Air and Stream Improvement, be conducted to build up a reliable body of knowledge on the amounts of specific volatile organics being discharged from pulp mill wastewaters and to determine the waste treatment systems that most effectively remove them. In particular, the following questions must be answered:

- Does the chemical composition of kraft paper mill wastewaters remain fairly consistent across large geographic areas or only within smaller geographic areas such as within the Southeast?
- How does the composition of these wastewaters vary with changes in wood and changes in the pulping process?
- Does the chemical composition of these wastewaters vary with season?
- What types of compounds are likely to build up in concentration if the wastewater is recycled?
- Can the in-plant source of the more refractory compounds be determined so that one or more small volume wastewater streams can be selected for special intensive treatment before they are combined with other in-plant wastewaters?

We recommend that other wastewater treatment systems be evaluated using the techniques developed for this study. In particular, the effectiveness of activated sludge and of activated carbon filters for reducing the concentrations of these organics would be useful information.

We also recommend that the information on specific organic pollutants contained in this report be used for developing programs to monitor for evidence of kraft paper mill pollution in receiving waters. Furthermore, some of the more abundant and/or resistant compounds (such as dehydroabietic acid, pimeric acid, isopimeric acid, sandaracopimeric acid, 13-abieten-18-oic acid, guaiacol, vanillin, stearic acid, oleic acid, margaric acid, alpha-terpineol, and camphor) should be considered as additions to measured parameters in future EPA effluent guidelines.

Often chronic and acute toxicity data are lacking for most of these compounds. We recommend that toxicologists examine these compounds for possible detrimental effects. We also recommend that taste and odor thresholds be determined, especially for the phenolics, where this information is lacking.

SECTION III

INTRODUCTION

A substantial body of knowledge exists concerning the identities of specific chemical compounds in various types and physical sections of trees and, to a lesser degree, in the wastewaters of paper mills. But little is known about what happens to these chemicals as they pass through wastewater treatment systems and enter into receiving waters. To our knowledge this study represents the first attempt to chemically characterize a wastewater, trace the dissolved volatile organics through a treatment system, and correlate this information with the traditional collective pollution parameter measurements (BOD, TOC). The results detailed in this report were gathered over a six-year period and portions of them have been presented or published previously.[1-5]

By tracing the chemicals through the treatment system one can identify which compounds are being effectively removed and which are resistant to the treatment in use. Any new chemicals produced during treatment are readily apparent. Once identifications are made, the approximate concentration of each compound can be calculated at each stage of the treatment. By comparing two or more different types of treatment, their effects on the individual compounds, or on the various classes of compounds in the wastewaters, can be ascertained. This knowledge will be particularly useful for advanced wastewater treatment and control studies, especially those involving wastewater recycling. A build-up in concentration of compounds resistant to the proposed treatment could be detrimental to closed-loop systems. If the segregated wastewater streams are analyzed before they are combined for treatment, the main sources of compounds resistant to treatment can be identified and singled out for more economical, specialized treatment, if desired or needed.

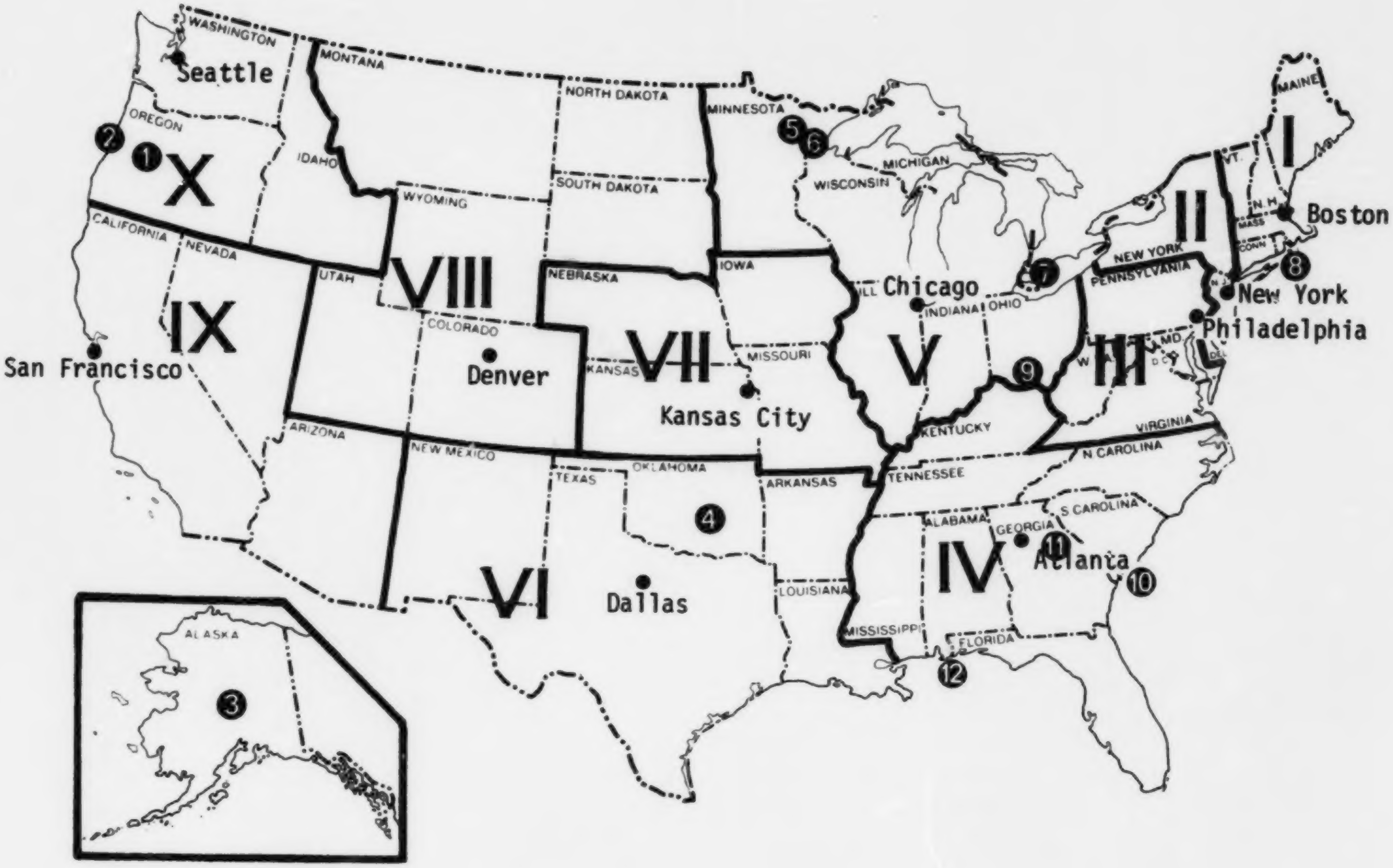
Knowledge of the specific chemical composition of treated wastewaters is also basic to the evaluation of the environmental impact of these wastewaters and to the problem of analyzing and controlling their discharge. Once these "refractory" compounds are identified, studies involving their fate and ecological effects can commence. Acute and, possibly more significant, chronic effects of these chemicals on aquatic life can be determined.

Ultimately, when more statistical data have been gained on the occurrence of specific "refractory" compounds in kraft paper mill wastewaters, and their environmental effects have been evaluated, recommendations relative to some of these compounds may be incorporated into the effluent guidelines for paper mill wastewater discharges. Information gained from these studies concerning specific organic contaminants has already been used by Region IV Surveillance and Analysis Division (SAD) in enforcement conferences[6,7] and is currently being used by Region VIII SAD[8] to determine if the source of some pollution problems lies with paper mill contaminants (Fig. 1). In the summer of 1972, we provided the Organic Analysis Unit of the Lower Mississippi River Field Facility with evidence that contaminants from paper mills in that area contributed measurably to the organic pollution of the Mississippi River.[9] Identification of specific resin acids and certain phenols in polluted waters provide strong evidence for contamination by paper mills.

This study should raise many questions and stimulate further work that will result in an accumulation of data from many other mills with other types of wastewater treatment. The report should be used as a tool by other laboratories that desire to study the composition of kraft pulp mill wastewaters and the effects of waste treatment on them. Although we relied heavily on gas chromatography-mass spectrometry (GC-MS) for compound identification, once the identifications are made and GC retention times are established, GC-MS is not absolutely necessary. A great deal of useful work can be accomplished with GC and standards of the compounds identified in this report. Most paper mill laboratories and college forestry or ecology laboratories have gas chromatographs available and the column packings used (carbowax 20M/TPA and SE-30) are very common ones.

PAPER MILL TREATMENT FACILITIES

Two mills, having similar processes but different waste treatments, were used in this study. The first, Paper Mill "A", daily produced about 1400 tons of containerboard in March 1972 when the samples were taken. Approximately 13 million gallons of water passed through the treatment system daily. Treatment consists of a primary clarifier, trickling biofilter, secondary clarifier, and 5 aerated lagoons with a total retention time of 2-2 1/2 days (Fig. 2). The trickling biofilter is 30 feet high, and 80 feet in diameter, and is packed with a vinyl core material having 97% void space. It handles an average daily load of 56,000 lb BOD and provides 50% BOD removal. The total BOD reduction through the whole system is reported to be in excess of 70%. In 1972, grab samples for chemical



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OKLAHOMA
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Figure 1 EPA Regional Districts

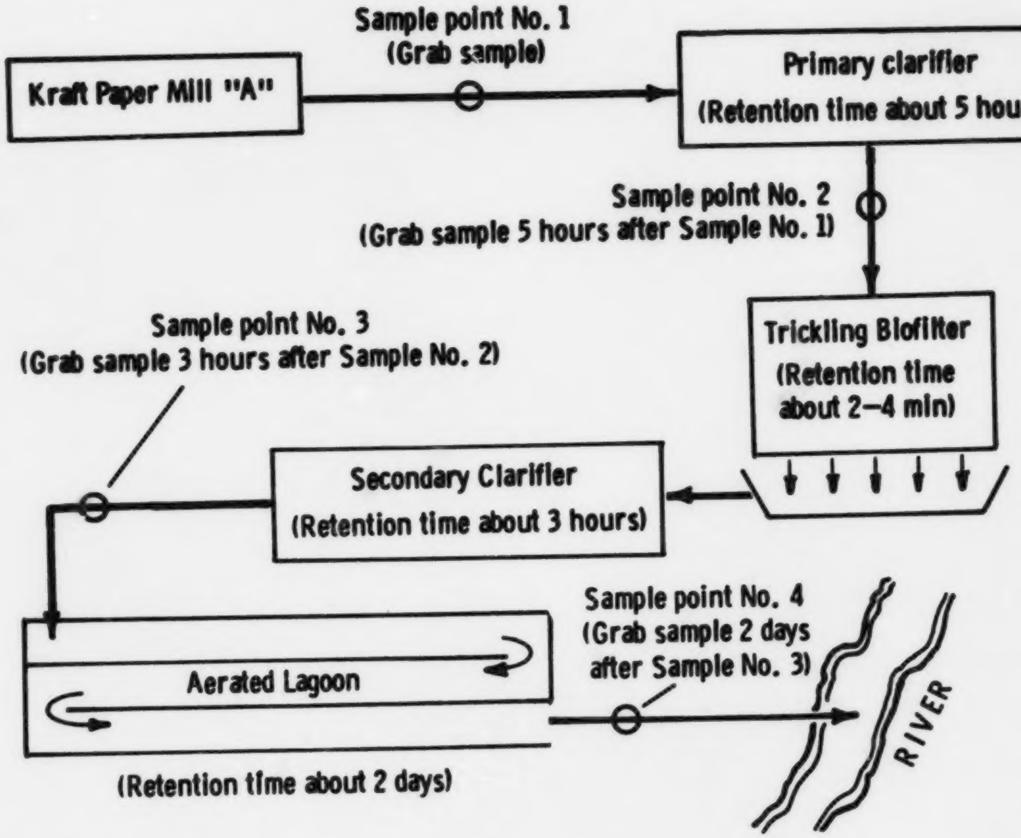

Sample point No. 1
(Grab sample)
Kraft Paper Mill "A"
Primary clarifier
(Retention time about 5 hours)
Sample point No. 2
(Grab sample 5 hours after Sample No. 1)
Trickling Biofilter
(Retention time about 2—4 min)
Sample point No. 3
(Grab sample 3 hours after Sample No. 2)
Secondary Clarifier
(Retention time about 3 hours)
Aerated Lagoon
(Retention time about 2 days)
Sample point No. 4
(Grab sample 2 days after Sample No. 3)
RIVER

Figure 2 Waste treatment diagram of Mill "A"

characterization were taken at various stages of the treatment system and time delays were programmed so that a "slug" of the effluent would be followed through the treatment facilities. However, quantitative analysis indicated that the slug was missed at the outfall. A second sampling period, in January 1974, used 3-day composites. Daily composites of the raw effluent from January 16 - 18 were combined. The quantitative results of these "average slugs" were much more satisfactory.

The second mill, Interstate Paper Corp. at Riceboro, Ga., daily produced about 540 tons of containerboard in March 1972 when the samples were taken. Approximately 5.5 million gallons of water passed through the treatment system daily. Treatment consists of lime addition at 0.1%, a 40-minute flocculation period followed by gravity clarification and a 650-acre (3-6 month retention) stabilization lagoon (Fig. 3). The highly alkaline effluent (pH 12) first undergoes partial neutralization to pH 10 by surface absorption of atmospheric carbon dioxide, accompanied by precipitation of nearly all the remaining calcium in the inlet section of the stabilization basin. Lime treatment removes about 90% of the color from the effluent. Overall BOD reduction is reported to be 93%, with a concentration of about 6 mg/l in the lagoon effluent.[10,11] The 1972 sampling consisted of 24-hour composites or grab samples at various stages of the treatment system as indicated in Figure 3.

During a second sampling period, in January 1974, 3-day composites were collected. Daily composites of the raw effluent and the outfall were collected from January 16-18. The raw effluent samples were combined, the outfall samples were combined, and the composites of each were analyzed.

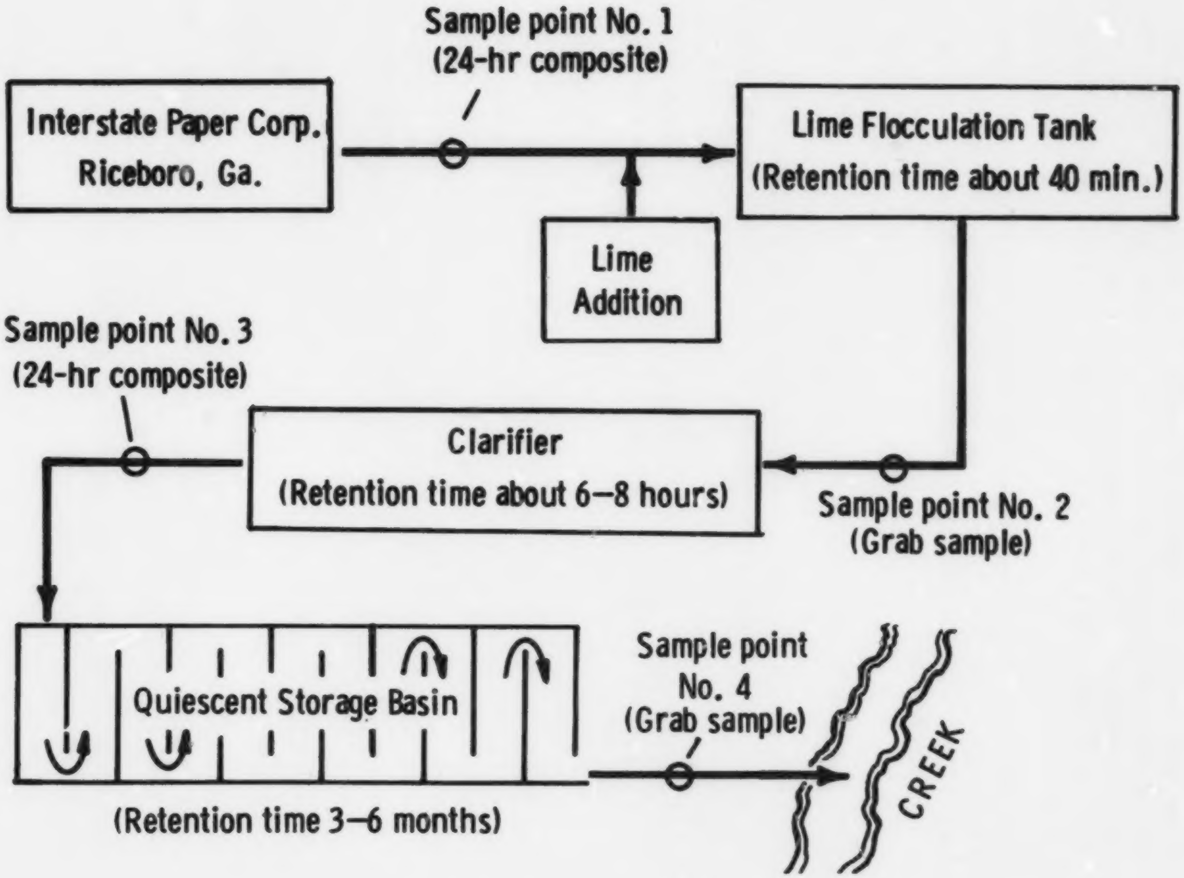

Sample point No. 1
(24-hr composite)
Interstate Paper Corp.
Riceboro, Ga.
Lime Flocculation Tank
(Retention time about 40 min.)
Lime
Addition
Sample point No. 3
(24-hr composite)
Clarifier
(Retention time about 6–8 hours)
Sample point No. 2
(Grab sample)
Quiescent Storage Basin
Sample point
No. 4
(Grab sample)
CREEK
(Retention time 3–6 months)

Figure 3 Waste treatment diagram of Interstate Paper Corp., Riceboro, Georgia

SECTION IV

ANALYTICAL PROCEDURES

SOLVENT EXTRACTION EFFICIENCIES

To determine the best of several common solvents used for liquid-liquid extractions, 1-liter portions of the Mill "A" aerated lagoon effluent were extracted with 400-ml portions of low boiling petroleum ether, diethyl ether, chloroform, and ethylacetate.

Each of the extracts was concentrated in a Kuderna-Danish evaporator (Fig. 4) and chromatographed under identical conditions. Chloroform was the best of the four solvents for these extractions.

CHLOROFORM VS CARBON CHLOROFORM EXTRACTS

Adsorption of organics on granulated carbon followed by extraction with chloroform provides a large amount of sample with which to work.

Accordingly, 1800 gal. of the Mill "A" aerated lagoon effluent was passed through a bank of 8 carbon filters in parallel. The carbon was dried and extracted with chloroform. The resulting extract was concentrated in a Kuderna-Danish apparatus, and chromatographed under conditions identical to those used for the 1-liter chloroform extract. Figure 5 shows a comparison of the two chromatograms. Most of the same peaks are present in both chromatograms. Although the relative intensity differs somewhat, the chromatograms are similar enough that, from a qualitative aspect, the two methods of sample concentration were essentially equivalent.

However, the carbon adsorption method has numerous disadvantages:

- Extent of adsorption varies with size of carbon particles, contact time with carbon (flow rate), and turbidity of the water.
- Carbons from various sources differ markedly in their adsorptive ability.
- A carbon column can go septic, causing biological degradation of the adsorbed organics.

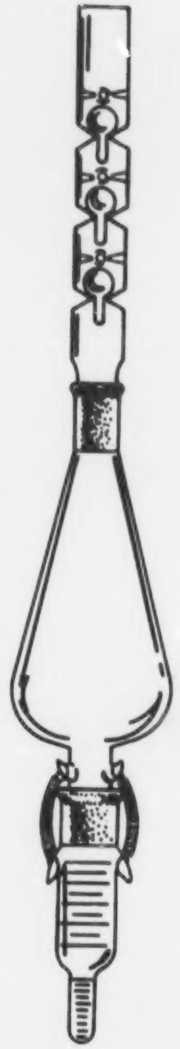


Figure 4 Kuderna-Danish concentrator

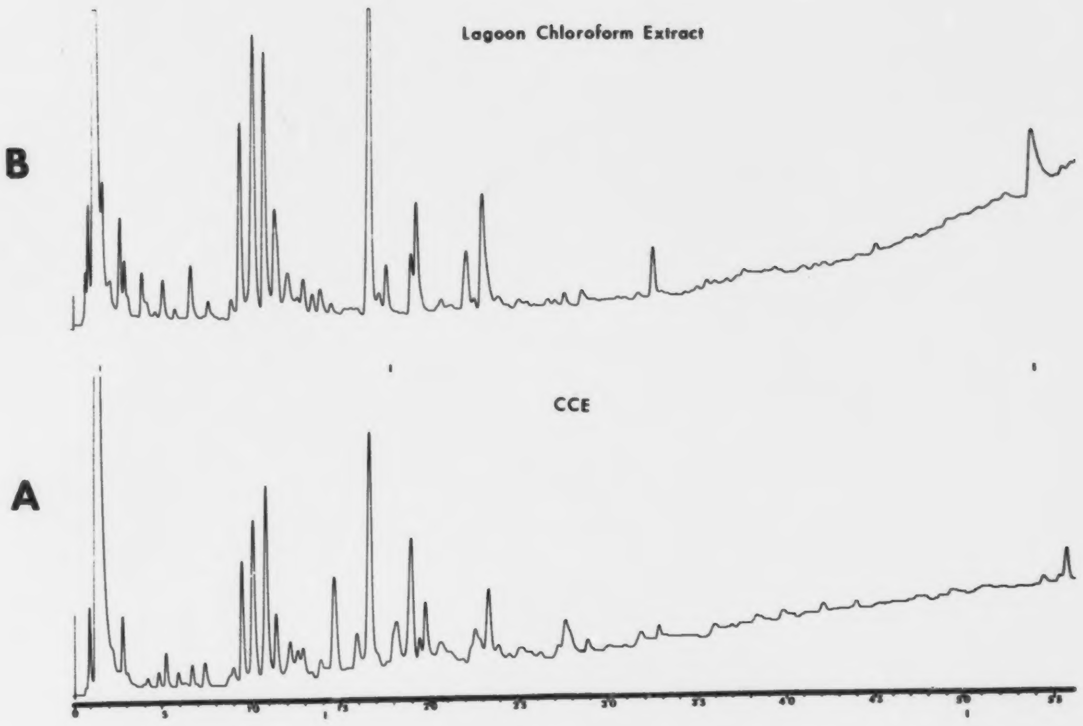


Figure 5 Gas chromatograms of the extracts of Mill "A" aerated lagoon effluent from (A) carbon chloroform extract (CCE) and (B) liquid-liquid chloroform extract.

- Even with the greatest practical contact times, not all organic matter in water will be adsorbed; highly polar compounds are not removed by carbon columns.

- It is more time-consuming to set up the pumps and equipment than it is to simply "grab" a sample for solvent extraction.

- Some compounds may be only partially desorbed from the carbon.

- A greater possibility of chemical change (isomerization, hydrolysis, etc.) exists when carbon with its large, active surface area is used.

We therefore elected to obtain the rest of our samples by the simpler technique of extraction with chloroform followed by Kuderna-Danish concentration.

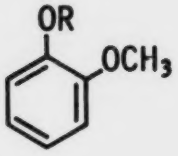
Papermill wastewater extracts contain two types of compounds: neutrals (predominately terpenes and their derivatives) and acidic compounds that are converted to their methyl derivatives to facilitate gas chromatographic (GC) separation.

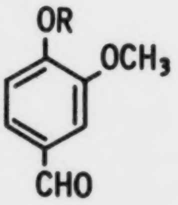
COMPARISON OF METHYLATION TECHNIQUES

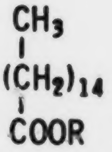
After the pH is raised to 11 and the neutrals are extracted, the aqueous solution can be methylated directly with dimethyl sulfate and sodium hydroxide, followed by extraction of the methyl derivatives with chloroform. This method has the advantage of being an *in situ* procedure; however, it is complex and time consuming. Alternatively, after extraction of the neutrals at a high pH, the solution can be made strongly acidic with concentrated hydrochloric acid,and the free acids and phenols extracted with chloroform. Methyl derivatives of the acids and phenols can then be made in a separate step using diazomethane, on-column GC methylation techniques, or several other common methylation procedures. An evaluation of each method was made using representative standard compounds and also using samples of kraft mill wastewater.

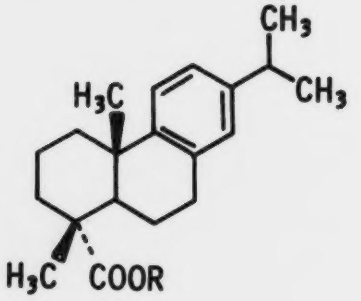
Methylation of Standards with Diazomethane

Ten milligrams each of guaiacol (I), vanillin (II), palmitic acid (III) and dehydroabietic acid (IV) (2 phenols, a saturated fatty acid, and a resin acid, respectively) were dissolved in 600 ml of water made to pH 11 with sodium hydroxide. The solution was placed in a separatory funnel, made acidic with concentrated hydrochloric acid, and extracted with three 133-ml portions of chloroform. After the combined extract was dried by passing it through a column of anhydrous sodium sulfate (previously baked at 600°C. for 2 hours to remove phthalate ester impurities), it was evaporated to near dryness in a Kuderna-Danish concentrator. Two ml of 10% methanol in ether was added and the sample was methylated by the standard procedure with diazomethane (Fig. 6). After methylation, chloroform was added to bring the volume to 10.0 ml, providing a theoretical 1,000 ppm solution of each of the four methyl derivatives based on 100% extraction and methylation. Duplicate injections into a gas chromatograph were made and the areas of the peaks (calculated by multiplying the peak height times the peak width at half height) were compared to the peak areas from standards of veratrole (V), veratraldehyde (VI), methyl palmitate (VII) and methyl dehydroabietate (VIII) (10 mg each) in 10 ml of chloroform (1,000 ppm). The calculated percent conversions were as follows: I → V, 18%; II → VI, 47%; III → VII, 70%; IV → VIII, 96%.









I: $R = H$
V: $R = CH_3$

II: $R = H$
VI: $R = CH_3$

III: $R = H$
VII: $R = CH_3$

IV: $R = H$
VIII: $R = CH_3$

Methylation of Standards with Dimethyl Sulfate

The reaction with dimethyl sulfate is dependent on temperature control, addition time, and reaction time, and on

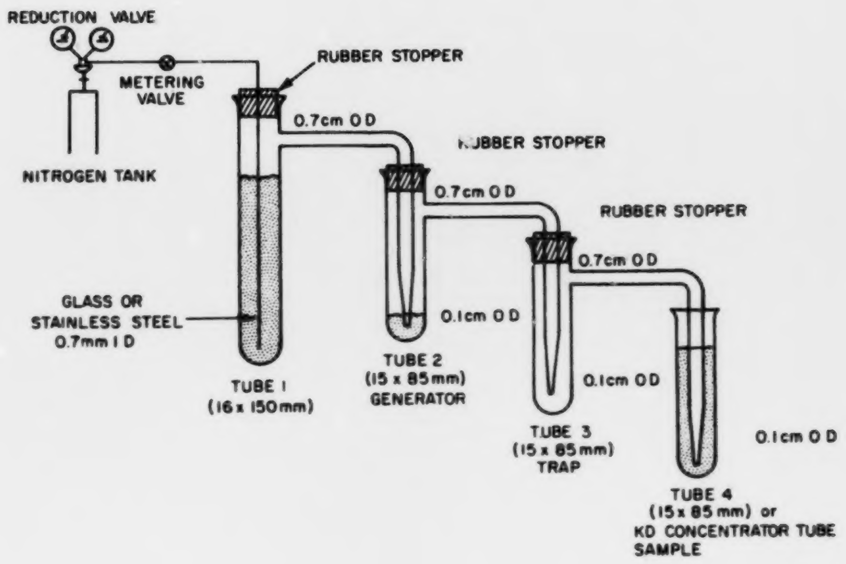

REDUCTION VALVE
METERING VALVE
NITROGEN TANK
RUBBER STOPPER
0.7cm O D
RUBBER STOPPER
0.7cm O D
RUBBER STOPPER
0.7cm O D
GLASS OR STAINLESS STEEL 0.7mm I D
0.1cm O D
0.1cm O D
0.1cm O D
TUBE 1 (16 x 150mm)
TUBE 2 (15 x 85mm) GENERATOR
TUBE 3 (15 x 85mm) TRAP
TUBE 4 (15 x 85 mm) or KD CONCENTRATOR TUBE SAMPLE

Figure 6 Apparatus for diazomethane methylation

how vigorously the reaction mixture is stirred. The procedure we use[16] is a variation of that described by Bicho *et al.*[17,18] Figure 7 shows a diagram of the apparatus. To determine the average yield and recovery of methylated model compounds from this reaction, four mixtures of I through IV were subjected to the same reaction and work-up procedures. Three mixtures containing 10 mg of each compound and a fourth mixture containing 25 mg of each compound were compared. After methylation, the products were extracted with chloroform, dried over sodium sulfate, and concentrated to less than 10 ml in a Kuderna-Danish apparatus. The volume was adjusted to 10.0 ml with chloroform. Comparision of GC peak areas with peak areas of standards V through VIII allowed calculation of the percent methylation and recovery. The results are summarized in Table I.

Methylation of Wastewater Samples with Dimethyl Sulfate

A 500-ml portion of the Mill "A" wastewater from sample point 2 (primary clarifier effluent) was made alkaline to pH 11 with sodium hydroxide and extracted with chloroform to remove the neutral compounds. Methylation of the aqueous layer with dimethyl sulfate was followed by re-extraction with chloroform to remove the methylated organics. After concentration in a Kuderna-Danish apparatus to 0.5 ml, 1.2 μl of the extract was chromatographed on a 50 ft support coated open tubular (S.C.O.T.) column coated with Carbowax 20 M/TPA and programmed from 100 to 200°C. at 4°/minute with an initial 2 minute hold at 100°. The chromatogram is shown in Figure 8-A. A control sample was prepared exactly the same way using 500 ml of distilled water; its chromatogram is shown in Figure 9-A.

Methylation of Wastewater Samples with Diazomethane

A 1-liter portion of the same wastewater was extracted with chloroform at pH 11 to remove neutral compounds and then made acidic to pH< 2 with conc. hydrochloric acid. The aqueous layer was re-extracted with four 200-ml portions of chloroform to remove the acids and phenols. The extracts were combined and divided into two equal portions, each representing 500 ml of the wastewater extract.

One portion was concentrated to near dryness in a Kuderna-Danish apparatus and methylated with diazomethane as previously described; the final volume was adjusted to 0.5 ml. The chromatogram of 1.2 μl of this sample under conditions identical to those of the previous sample is shown in Figure 8-B. A control sample was prepared from 400

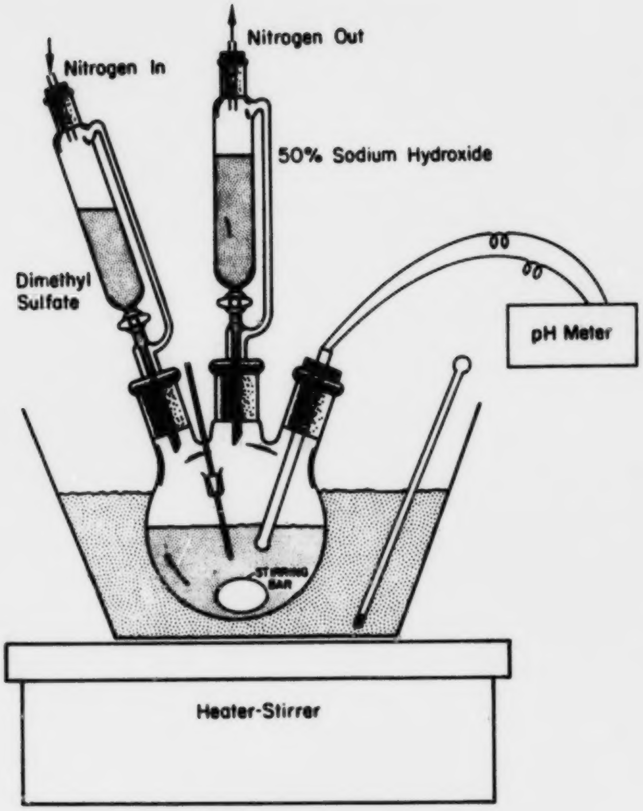

Nitrogen In
Nitrogen Out
50% Sodium Hydroxide
Dimethyl Sulfate
pH Meter
STIRRING BAR
Heater-Stirrer

Figure 7 Apparatus for dimethyl sulfate methylation

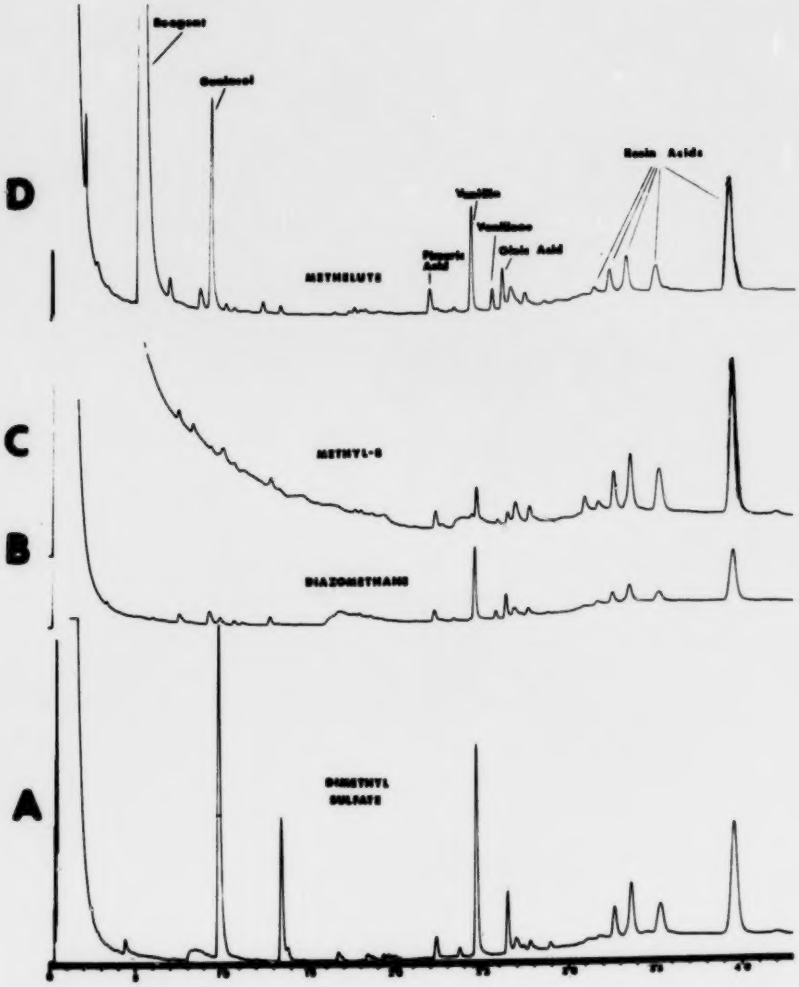


Figure 8 Chromatograms of the methyl derivatives from a kraft paper mill wastewater sample using (A) dimethyl sulfate, (B) diazomethane, (C) Methyl-8, and (D) MethElute as methylating reagents

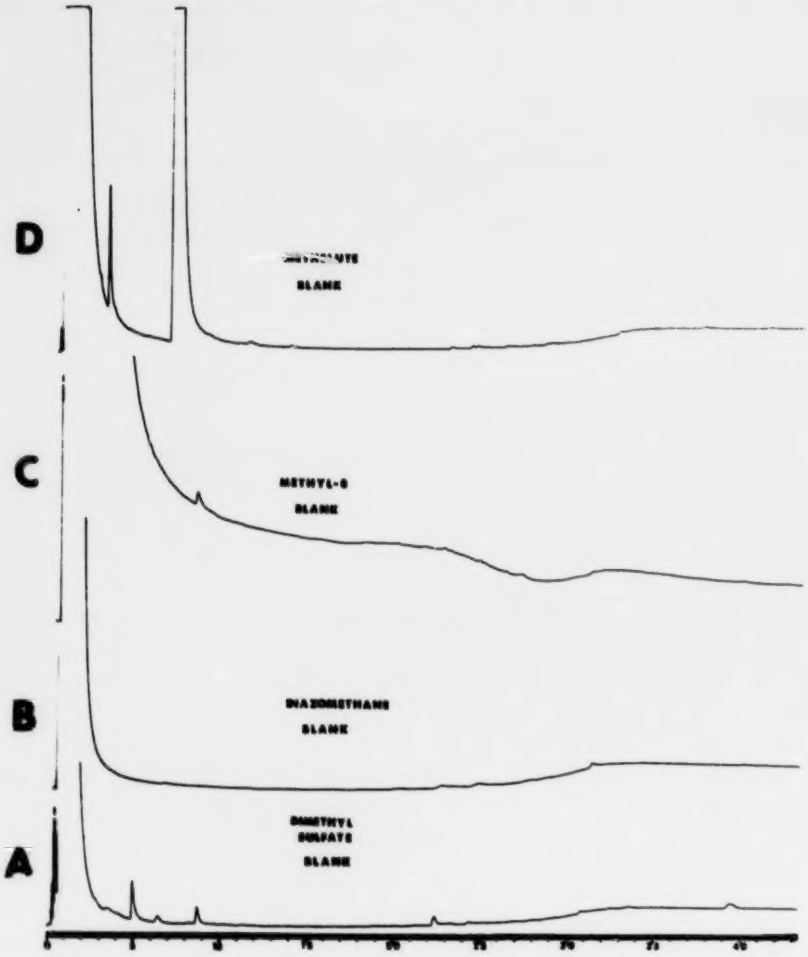


Figure 9 Chromatograms of control samples treated with (A) dimethyl sulfate, (B) diazomethane, (C) Methyl-8, and (D) MethElute as methylating reagents

Table 1

PERCENT METHYLATION AND RECOVERY OF MODEL PHENOLS AND ACIDS USING DIMETHYL SULFATE

Reaction No.	I → V	II → VI	III → VII	IV → VIII
#1 (10 mg each)	74%	60%	47%	39%
#2 (10 mg each)	91%	76%	64%	57%
#3 (10 mg each	100%	81%	66%	84%
#4 (25 mg each)	96%	69%	53%	60%
AVERAGE	90%	72%	58%	60%

ml of chloroform subjected to the same prodecure; the chromatogram of 1.2 µl of the control is shown in Figure 9-B.

Methylation of Wastewater Samples with On-Column Reagents

The second portion of the chloroform extract was concentrated in a Kuderna-Danish apparatus to near dryness and MethElute (trimethylanilinum hydroxide in methanol; Pierce Chemical Co.) was added to bring the volume to 0.5 ml. The chromatogram of 1.2 µl of this sample under conditions identical to those of the previous two samples is shown in Figure 8-D. MethElute provides on-column methylation of the sample. A control sample was prepared from 400 ml of chloroform subjected to the same procedures. The chromatogram of 1.2 µl of the control is shown in Figure 9-D.

Another 500-ml portion of the wastewater, after extraction of neutral compounds at pH 11, was made acidic and extracted with four 100-ml portions of chloroform. The extracts were combined, dried, and concentrated to near dryness as before in a Kuderna-Danish apparatus. Enough Methyl-8 (DMF dimethyl acetal in pyridene; Pierce Chemical Co.) was added to bring the volume to 0.5 ml and the solution was heated at 60°C for 15 minutes in a reacti-vial. The chromatogram of 1.2 µl of this sample, under conditions identical to those of the other three, is shown in Figure 8-C. A control sample was prepared from 400 ml of chloroform subjected to the same procedure. The chromatogram of 1.2 µl of the control is shown in Figure 9-C.

Methylation Evaluation

Comparison of the chromatograms in Figure 8 shows that dimethyl sulfate and MethElute are better reagents for methylating these samples than diazomethane or Methyl-8. Phenols, especially guaiacol, were not methylated well with diazomethane or Methyl-8. Although extraction of guaiacol and other phenols with chloroform is not 100%, incomplete extraction can be eliminated as a major contributor to the smallness of the guaiacol peak in the diazomethane sample because the samples for diazomethane and MethElute treatment came from the same extract, which was divided into two portions, and the MethElute sample shows a large veratrole peak.

The dimethyl sulfate appears to be equivalent to MethElute with respect to methylation of the resin and fatty acids.

However, the larger phenolic peaks in the dimethyl sulfate sample and absence of the MethElute reagent peak favored use of dimethyl sulfate for all remaining methylations involved in analysis of these wastewaters.

INSTRUMENTATION

Gas Chromatograph

Most of the chromatograms shown in this report were obtained with a Varian 1400 gas chromatograph (GC) equipped with a flame ionization detector (FID). Because helium is usually used as a carrier gas with gas chromatographs interfaced with mass spectrometers (GC-MS), we used helium as a carrier gas routinely in developing our chromatographic conditions. Generally, a commercially prepared (Perkin-Elmer) 50-ft support coated open tubular (S.C.O.T.) capillary column coated with carbowax 20M/terephthalic acid (K 20 M/TPA) was used for separation. The optimum carrier gas flow for our GC-MS systems operating under a vacuum and using a Gohlke jet separator is 16 to 18 ml/minute. If a helium flow of about half this is used for optimizing conditions with an auxillary GC (operating under atmospheric pressure), the other chromatographic variables (temperature program rate, initial temperature, initial hold) will hold true when the same column is transferred to the GC-MS system.

GC-MS-Computer System

From 1968 to 1971, a Perkin-Elmer/Hitachi RMU-7 double focusing mass spectrometer connected to a Perkin-Elmer 900 GC through a Watson-Bieman separator was used to analyze the sample extracts from kraft papermill wastewaters. Spectra acquisition, data reduction, and spectral interpretation had to be done manually. Despite these limitations, much of the early work reported in this project[1-4,6,7] was accomplished with this system.

Now, a semi-automatic GC-MS-computer system is used for pollutant identification. The GC is a modified Varian 1400 chromatograph with a temperature-controlled oven that can be programmed from 50° to 500° C. It has no independent detector and serves only as a specialized inlet to the mass spectrometer. An all-glass, single-stage Gohlke jet separator enriches organic samples by utilizing differences in diffusion rates of sample and carrier gases in a turbulent jet.

The Finnigan 1015 quadrupole mass spectrometer with three mass ranges extending to m/e 750 is capable of unit resolution throughout the range. At a scan speed of 120 amu/sec, sensitivity is adequate to give identifiable spectra for 20 ng of material introduced into the GC inlet. The liquid inlet is used for introduction of calibration compounds, the direct probe for solid materials.

A System Industries interface (analog-to-digital converter, and the digital-to-analog converter) permits the Digital Equipment Corporation (DEC) PDP8/e computer to control the mass spectrometer during calibration and data acquisition; to accept data from the mass spectrometer; and to control a Houston plotter during data reduction.

The DEC PDP8/e computer has a 4096 word core and an ASR33 teletypewriter. Programs, raw data, and reduced data are stored on either two DECtape units or a Diablo disc. Output of the reduced data is achieved under computer control via the plotter, the teletypewriter, or an acousticoupler. The coupling device connects the PDP8/e to the CDC 6400 computer at Battelle Laboratories, Columbus, Ohio, and permits semi-automatic spectrum identification by a matching program, fully described elsewhere.[19,20]

Using this system, data reduction times are much less than for the manual reduction methods formerly used. About 2 hours of operator time is required to output reduced data for an 80-peak chromatogram. Data output time ranges from slightly more than four hours for the disc system to more than eight hours for the tape system. Manual data reduction and spectral plotting of the same 80 chromatographic peaks would require several weeks.

SECTION V

IDENTIFICATION OF ACIDS AND PHENOLS

The acids and phenols in each wastewater were analyzed as a group for both the 1972 samples and the 1974 samples. Results were then compared. The neutral extractable organics (mostly terpenes) were analyzed separately and are discussed in the next section.

GC-MS ANALYSES

Each of the 8 samples (4 from each mill at various sampling points) was methylated with dimethyl sulfate as previously described. Following this, each of the 8 extracts was analyzed by GC-MS using the Finnigan 1015 GC-MS computer system. After a sample is injected into the GC, the mass spectrometer automatically scans its pre-set mass range every five seconds (or other pre-set interval). As it does so, the plotter draws a trace that is equivalent to a GC signal. Since our samples were pre-analyzed on an auxiliary GC, the auxiliary chromatograms were used to determine when to terminate the GC-MS runs. When each run is complete, the computer plots a reconstructed gas chromatogram (RGC). The RGC peaks are all normalized to the amplitude of the largest peak, arbitrarily plotted at 100. Each point on the spectrum number scale under the RCG represents a complete mass spectrum. Figure 10-A shows the RGC of the methylated acids and phenols from the lagoon wastewater of Mill "A". Any of the spectra can be displayed individually; therefore, by choosing the appropriate spectrum numbers, the analyst can see the composition of each peak in the chromatogram.

Specialized techniques of MS or data reduction can be used to detect a specific material or class of materials in a mixture. The most common technique is the generation of the limited mass reconstructed gas chromatogram (LMRGC). For example, in Figure 10-A, the RGC shows as peaks those spectra that contain significant numbers of any ion fragments of m/e 33 to 450. Below the RGC is the LMRGC in which the computer was instructed to respond only to those spectra that contain the m/e 149 fragment (Figure 10-B). This fragment is usually due to protonated phthalic anhydride (IX), found in the spectra of phthalate esters. The LMRGC, therefore, indicates that two of the sample peaks, 44 and 61, are phthalates. The numbers 44 and 61 refer to the compound designations listed in Table 2.

Table 2. ACIDS AND PHENOLS IDENTIFIED IN BOTH KRAFT PAPER MILL EFFLUENTS WITH APPROXIMATE CONCENTRATIONS

Peak No.	Compound Identified as Methyl Derivative (Parent Compound)	Confirmed By	Approximate Conc. in mg/1 Mill "A" Sample Points 1	2	3	4	Approximate Conc. in mg/1 Interstate Sample Points 1	2	3	4
1	Furfuryl methyl ether (furfuryl)	GC	0.010	0.005	--	--	0.010	--	--	--
2	Anisole (Phenol)		0.006	--	--	--	0.005	0.005	--	--
3	1,3-Dimethoxy-2-propanol		--	--	--	--	--	0.010	--	--
4	Methyl trisulfide		--	0.005	--	--	--	--	--	--
5	Unidentified--apparent MW=103		0.035	0.035	0.040	0.035	0.025	0.055	0.020	0.015
6	Benzaldehyde	GC	0.002	--	--	--	0.005	--	--	--
7	Dimethylsulfoxide	GC	--	--	0.055	--	0.010	0.080	0.020	0.020
8	Ethyl carbamate	GC	0.010	0.015	0.025	0.020	--	0.080	0.020	0.010
9	Borneol	MS,GC	--	--	--	--	0.010	0.020	0.060	--
10	α-Terpineol	MS,GC	0.020	0.002	0.001	--	0.005	0.020	0.115	0.002
11	Veratrole (Guaiacol)	MS,GC	2.700	2.400	0.360	0.170	2.200	3.200	3.700	0.035
12	o-Nitrotoluene	MS,GC	--	--	--	0.015	--	--	--	--
13	Methyl o-hydroxybenzoate (o-hydroxybenzoic acid)		0.010	0.005	--	--	--	--	--	--
14	Methyl mandelate (mandelic acid)		--	--	--	0.025	--	--	--	--
15	Dimethylsulfone	GC	0.240	0.400	0.400	0.055	0.240	0.540	0.400	0.130
16	Unidentified aromatic MW=166		0.130	0.090	0.050	0.005	0.050	0.030	0.160	--
17	Methyl isomyristate (C_{14} fatty acid)	GC	--	--	--	0.003	--	--	--	0.001
18	p-Methoxybenzaldehyde (p-hydroxybenzaldehyde)		0.055	0.050	0.020	0.025	0.090	0.240	0.190	0.005
19	Eugenol		0.025	0.025	0.025	--	--	--	--	--
20	Methyl myristate (C_{14} fatty acid)	MS,GC	--	--	--	0.020	--	--	--	0.010
21	Unidentified aromatic, MW=196		--	--	0.045	0.035	--	--	--	--
22	Unidentified nonaromatic, MW=196		--	--	--	--	0.170	0.175	0.245	0.065
23	Methyl anteisopentadecanoate (C_{15} fatty acid)	MS,GC	--	--	0.010	0.020	--	--	--	0.005
24	Methyl 10-methyltetradecanoate (C_{15} fatty acid)		--	--	--	0.030	--	--	--	0.002
25	p-Methoxyacetophenone (p-hydroxyacetophenone)	GC	0.020	0.025	0.030	--	0.060	0.130	0.080	0.010
26	Methyl pentadecanoate (C_{15} fatty acid)	MS,GC	--	--	--	0.020	--	--	--	0.010

Table 2 (continued). ACIDS AND PHENOLS IDENTIFIED IN BOTH KRAFT PAPER MILL EFFLUENTS WITH APPROXIMATE CONCENTRATIONS

Peak No.	Compound Identified as Methyl Derivative (Parent Compound)	Confirmed By	Approximate Conc. in mg/l Mill "A" Sample Points 1	2	3	4	Approximate Conc. in mg/l Interstate Sample Points 1	2	3	4
27	Methyl isopalmitate (C_{16} fatty acid)	MS,GC	--	--	0.015	0.030	--	--	--	0.003
28	Methyl palmitate (C_{16} fatty acid)	MS,GC	0.070	0.190	0.180	0.430	0.140	0.160	0.035	0.017
29	Methyl palmitelaidate (C_{16} trans-9-unsaturated fatty acid)	MS,GC	0.005	0.020	0.025	0.125	--	--	--	0.010
30	Ethyl palmitate (C_{16} fatty acid)	MS,GC	--	--	--	0.030	--	--	--	--
31	Methyl anteisomargarate (C_{17} fatty acid)	MS,GC	0.020	0.025	0.040	0.050	0.030	0.120	0.040	0.001
32	Methyl 3,4-dimethoxyphenylacetate (Homovanillic acid)		0.050	0.075	0.090	0.080	0.090	0.365	0.150	0.003
33	Veratraldehyde (Vanillin)	MS,GC	1.500	1.700	0.450	0.410	2.100	4.400	2.600	0.070
34	2-Methylthiobenzothiazole (2-Mercaptobenzothiazole)	GC	0.035	0.025	0.030	0.025	--	--	--	--
35	Methyl vanillate (Vanillic acid)	GC	--	--	0.005	0.005	--	--	--	--
36	Acetoveratrone (Acetovanillone)	GC	0.420	0.490	0.450	0.370	0.820	1.600	0.860	0.120
37	Methyl stearate (C_{18} fatty acid)	MS,GC	0.025	0.065	0.045	0.110	0.100	0.100	0.020	--
38	Methyl oleate (C_{18} cis-9-unsaturated fatty acid)	MS,GC	0.470	0.600	0.430	0.400	0.570	0.510	0.120	0.080
39	3,4,5-Trimethoxybenzaldehyde (Syringaldehyde)	MS,GC	0.070	0.070	0.070	0.040	0.070	0.100	0.070	0.020
40	Methyl linoleate (C_{18} cis,cis-9, 12-diunsaturated fatty acid)	MS,GC	0.350	0.470	0.230	0.100	0.450	0.920	0.160	--
41	3,4-Dimethoxypropiophenone (3-Methoxy-4-hydroxypropiophenone)	GC	0.060	0.080	0.025	--	--	--	--	--
42	3,4-5-Trimethoxyacetophenone (Acetosyringone)	GC	0.055	0.060	0.050	0.050	0.090	0.200	0.130	0.005
43	Methyl Arachidate		0.030	0.035	0.030	0.025	0.035	0.025	--	--
44	a Dihexylphthalate		--	--	--	--	--	--	--	--
45	Unidentified resin acid "A" methyl ester		--	--	--	--	0.070	0.075	0.130	0.020
46	Unidentified resin acid "B" methyl ester		0.030	0.055	0.095	0.100	--	--	--	--

Table 2 (continued). ACIDS AND PHENOLS IDENTIFIED IN BOTH KRAFT PAPER MILL EFFLUENTS WITH APPROXIMATE CONCENTRATIONS

Peak No.	Compound Identified as Methyl Derivative (Parent Compound)	Confirmed By	Appropriate Conc. in mg/l Mill "A" Sample Points 1	2	3	4	Approximate Conc. in mg/l Interstate Sample Points 1	2	3	4
47	Unidentified resin acid "C" methyl ester		0.100	0.055	0.010	--	--	--	--	--
48	Unidentified resin acid "D" methyl ester		--	--	--	--	--	--	--	0.145
49	Unidentified unsaturated fatty acid methyl ester		--	--	0.005	0.035	--	--	--	--
50	Methyl pimerate (resin acid)	MS,GC	0.245	0.475	0.570	0.800	1.270	0.825	0.610	0.500
51	Methyl sandaracopimerate (resin acid)	MS,GC	0.050	0.060	0.125	0.045	0.340	0.245	0.275	0.110
52	Unidentified resin acid "E" methyl ester		0.025	--	--	--	--	--	--	--
53	Methyl-13-Abieten-18-oate (resin acid)	MS,GC	--	0.430	0.800	1.400	0.100	0.035	0.050	1.050
54	Unidentified unsaturated fatty acid methyl ester similar to araconidate		0.035	--	--	--	0.075	--	--	--
55	Methyl isopimerate (resin acid)	MS,GC	0.430	0.660	0.770	0.780	4.400	1.700	1.200	0.800
56	Methyl abietate (resin acid)	GC	0.370	0.430	0.420	0.050	3.300	1.500	1.900	--
57	Methyl dehydroabietate (resin acid)	MS,GC	1.500	1.300	4.000	1.000	3.300	2.700	3.600	3.900
58	Methyl 6,8,11,13-Abietatetraen-18-oate (resin acid)	MS,GC	0.065	0.070	0.170	0.095	0.160	0.115	0.280	0.180
59	Methyl neoabietate (resin acid)	MS,GC	0.105	0.125	--	--	1.300	1.200	0.450	--
60	Methyl lignocerate (C_{24} fatty acid)	MS,GC	--	--	0.055	0.030	--	--	--	--
61	a Dioctylphthalate		--	--	--	--	--	--	--	--
Total			9.380	10.692	10.246	7.123	21.690	21.480	17.700	7.243

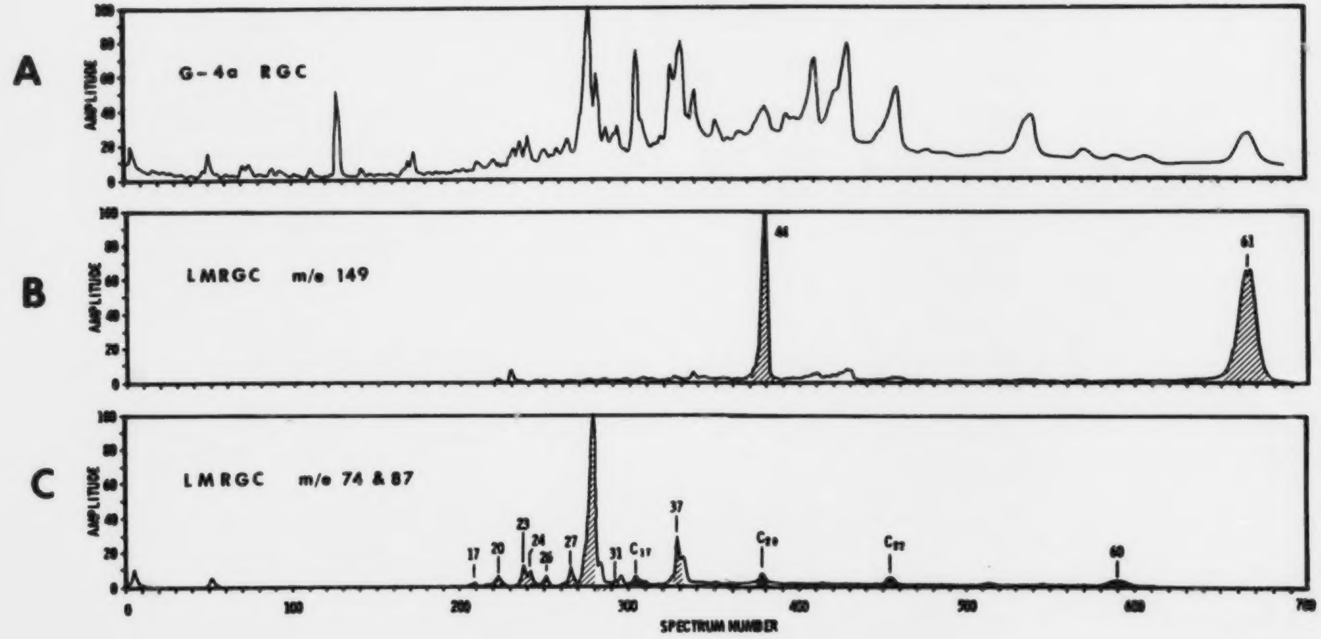
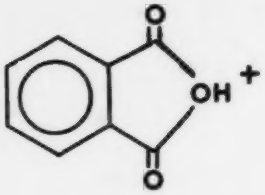
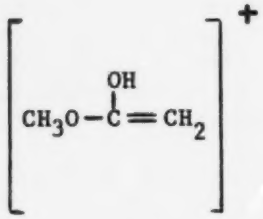


Figure 10 Computer reconstructed (A) gas chromatogram, (B) LMRGC using m/e 149 and (C) LMRGC using m/e 74 and 87 superimposed on the same axis

Similar characteristic fragments exist for other classes of compounds. The most useful ones in this study were m/e 74 and 87, which come from fragments X and XI respectively and are characteristic of long-chain saturated fatty acid methyl esters.[21]

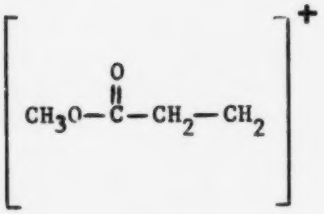


IX



$$\left[CH_3O-\overset{OH}{\overset{|}{C}}=CH_2\right]^+$$

X



$$\left[CH_3O-\overset{O}{\overset{\|}{C}}-CH_2-CH_2\right]^+$$

XI

The LMRGC corresponding to these fragments for the lagoon wastewater from Mill "A" is shown in Figure 10-C. The numbers above the shaded peaks correspond to the identifications in Table 2. The 3 shaded peaks probably correspond to methyl margarate (C-17), methyl arachidate (C-20), and methyl behenate (C-22), but, because these were all minor components of unresolved peaks, the mass spectra were not definite enough to confirm their identification. Margarate and arachidate were later confirmed in a 1974 sample of these wastewaters. Arachidate is assigned peak number 43 in Table 2.

We have found that using the m/e 74 and 87 characteristic fragments together results in a more reliable indication of long-chain saturated fatty acid methyl esters than either one by itself. The two LMRGC's are superimposed and different colored pens are used for each trace. The peaks at spectrum number 6 (m/e 87 search) and at spectrum number 51 (m/e 74 search) contain only one of the characteristic fragment ions and are therefore not long-chain fatty acid methyl esters.

From the RGC and/or LMRGC traces, spectra of interest are chosen for output. The mass spectrum can be either plotted or tabulated. This spectrum will be that of the sample compound plus various fragments from GC column bleed, traces of air, oil, and moisture, and possibly residual material from a previously eluted compound. In the worst cases the GC peaks may be incompletely resolved so that the mass spectrum contains not only the fragments of interest but also fragments from other compounds comprising the peak envelope.

An example of this situation is shown in Figure 11-A. Some of the background fragments, from acetoveratrone and methyl oleate, mask fragment peaks of the fatty acid methyl ester mass spectrum. Computer subtraction of the appropriate quantities of these impurities, indicated by an examination of the RGC (0.6 of the mass spectrum of acetoveratrone and 0.2 of the mass spectrum of methyl oleate), produced the mass spectrum shown in Figure 11-B. This spectrum is now clearly recognizable as that of a saturated fatty acid methyl ester, since the base peak is at m/e 74 and the second most intense fragment occurs at m/e 87.

The mass spectrum is a chemical fingerprint that is characteristic of a compound and can be interpreted to give the structure of an unknown compound. Another way to identify an unknown compound is to match its mass spectrum with that of a known compound from a library of mass spectra. We employ both methods; but to minimize the time spent identifying the hundreds of spectra resulting from the GC-MS data output from complex mixtures such as these extracts, we first execute computer matching of the spectra. This is followed by a manual comparison of the computer matched spectra with the unknown mass spectra.

Widespread use of GC/MS/spectra-matching in pollutant identification requires easy access to a central spectra library,[19] rapid matching, and an indication of the similarity of the unknown spectrum to the reference spectrum for each match. An EPA program that provides such information was developed using the algorithm of a matching program described in the literature.[22] This rapid program was developed jointly by Battelle and the Southeast Environmental Research Laboratory, and utilizes a CDC 6400 time-shared computer.[23]

An application of the matching program is shown in Figure 11-B. The computer match output, inset on the spectrum, shows that there were a total of 62 hits. The five best matches are printed with the best match (correctly identified as methyl stearate) listed first and the others in descending order. The "similarity index" (SI) gives the user an immediate indication of the quality of the matches. The SI will show whether it is a poor match (<0.2 if the data base does not contain any closely related compounds), one of several fair matches (0.2-0.35 if the correct compound is not in the data base but related ones are), or a good match (>0.35 if the SI of the second best hit is significantly lower).

Additional information provided with the match shows (in sequence after the chemical name) the source of the mass

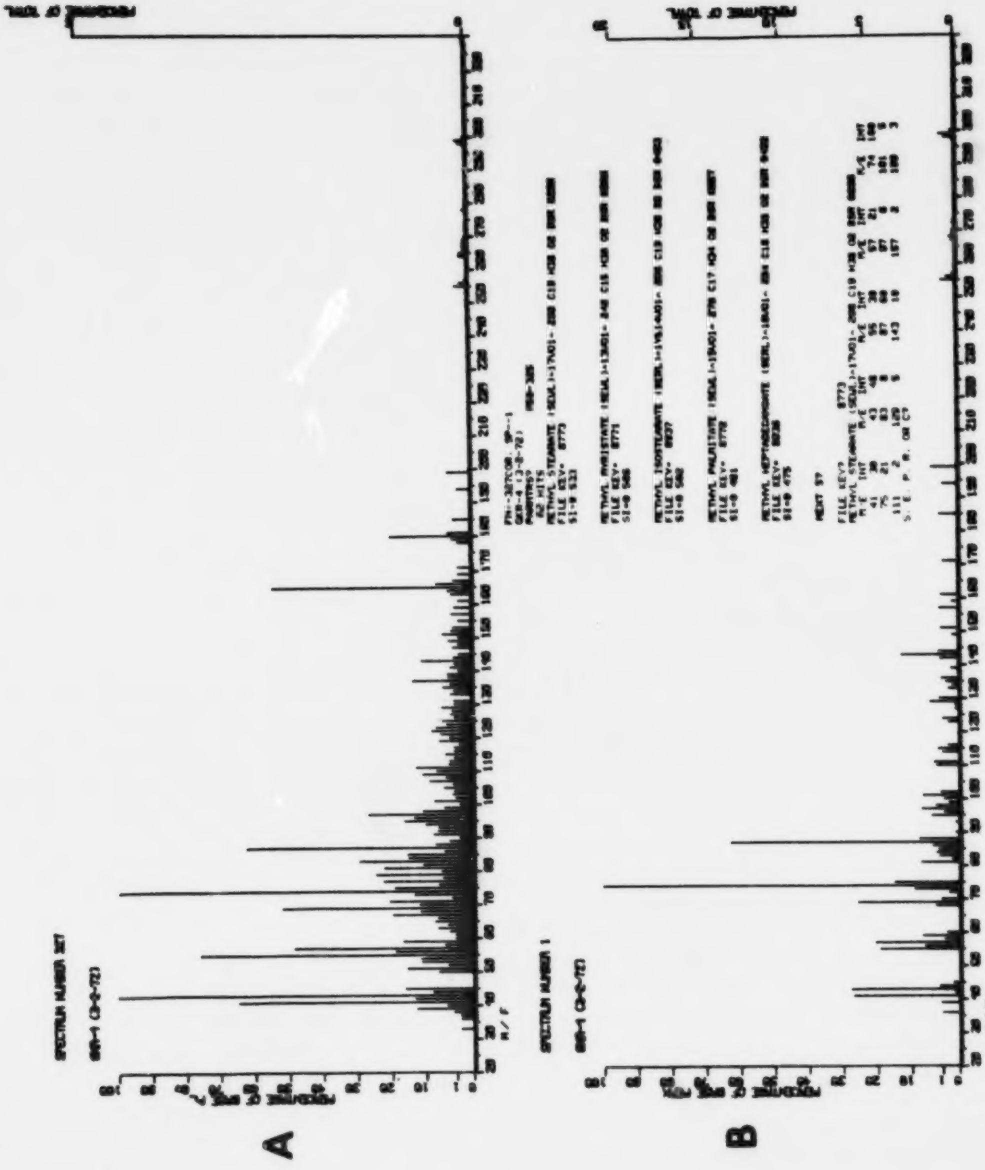


Figure 11 Mass spectra of (A) methyl stearate without impurity and background subtraction and (B) methyl stearate after computer subtraction of mass fragments from interfering compounds on either side of it

spectral data (SERL indicates the Southeast Environmental Research Laboratory), the Wiswesser Line Notation, the molecular weight, the empirical chemical formula, the Battelle spectrum number, and the file key. A computer print-out of the spectral data used for the match can be obtained with the file key number. The spectral data from file key 8773 is also inset on the spectrum in Figure 12-B. The match of these data (the 2 most intense fragments every 14 mass units) with the plotted spectrum is good. The other matches, in decreasing order of SI number and in groups of 5, would also be printed out if desired.

ANALYSIS OF THE 1972 SAMPLES

All compounds identified in the acidic fractions of both mills' wastewater samples in 1972 are listed in Table 2. The peak numbers assigned are used throughout this report. The approximate concentrations for each compound are calculated in milligrams per liter (mg/l) based on the sample peak area relative to the peak area and flame response of four representative compounds: veratrole, 3,4-dimethoxybenzaldehyde, methyl palmitate, and methyl dehydroabietate. Corrections were not made for inefficiency in methylation and recovery (70-90% with phenols; 60% with fatty and resin acids) in the calculated concentrations. Consequently, these values are lower than they may actually be.

Fatty Acids

A total of 17 different fatty acids were found in the wastewaters of both mills; 15 were identified and two unsaturated acids remain unidentified.

The fatty acid content of both raw wastewaters (sample point 1) were qualitatively similar. Seven of the eight fatty acids present in the raw wastewaters were found at both mills (palmitic, anteisomargaric, stearic, oleic, linoleic, arachidic, and unidentified unsaturated acid #54).

More dissimilarity existed in the fatty acid content of the two lagoon effluents (sample point 4). Ten acids were found in both effluents--isomyristic, myristic, anteisopentadecanoic, 10-methyltetradecanoic, pentadecanoic, isopalmitic, palmitic, palmitelaidic, anteisomargaric, and oleic. Six others were found in the lagoon effluent of one mill but not the other--ethyl palmitate (as the ester), stearic, linoleic, lignoceric, arachidic, and unidentified unsaturated acid #54. In both mill wastewaters a greater number of fatty acids was found in the lagoon effluent than in the influents. The majority of these new compounds are saturated low molecular weight (C-14, C-15, C-16) branched and straight-chain fatty acids. They are probably metabolites of aquatic life in the lagoons.

Significance of Fatty Acids--

Fatty acids are the main components of the parenchyma cell resin of both hardwoods and softwoods and occur mainly as glyceride esters.[24] Although those with even numbers of carbons are present in larger amounts than those with odd numbers of carbons, all the fatty acids from C-12 to C-21 have been found. Recent work identified 30 fatty acids in tall oil from pine and birch including odd-numbered carbon fatty acids and one branched fatty acid.[25] Fatty acids are therefore expected to be in abundance in the raw effluents from kraft mills.

Fatty acids are not generally considered to be very toxic although the California "Water Quality Criteria" lists the minimum lethal dose (MLD) for several of them at 5.0 mg/l for fish.[26] Recent work by Leach and Thakore[27] has indicated that the sodium salts of unsaturated fatty acids are more toxic to fish than those of saturated fatty acids. Furthermore, among the C_{18} unsaturated fatty acids, the toxicity of the sodium salts increased with increasing unsaturation in the order oleic<linoleic<linolenic (oleic has 1, linoleic has 2, and linolenic has 3 double bonds).

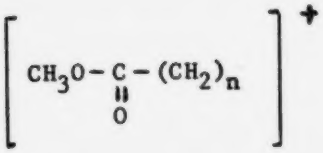
Little or no work has been reported on the analyses of fatty acids in treated paper mill effluents. We have found no previous report of odd-numbered carbon and branched fatty acids in treated wastewaters. The presence of 10-methyltetradecanoic acid and ethyl palmitate, although in small amounts, is unexpected.

Methyl 10-Methyltetradecanoate--

The only fatty acid identification not verified by comparison of GC retention time with a standard is the methyl ester of 10-methyltetradecanoate. Its identification rests solely on interpretation of its mass spectrum.

Mass spectra of saturated unbranched fatty acid methyl esters contain, in addition to m/e fragments 74 and 87 discussed previously, ionized fragments corresponding to structure XII formed by simple cleavage along the chain.[21]

The mass spectrum of the peak identified as methyl palmitate is a good example of this fragmentation (Figure 12-A).



$$\left[CH_3O-\underset{\substack{\| \\ O}}{C}-(CH_2)_n \right]^+$$

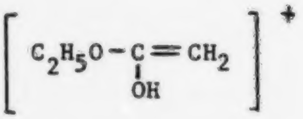
XII

Introduction of a methyl group in the carbon chain causes changes in the fragmentation pattern because of easy cleavage alpha to the tertiary carbon atom.[28] This results in a gap of 28 mass units when the CH_3-CH- moiety is cleaved. Figure 12-B shows the mass spectrum of the compound tentatively identified as methyl 10-methyltetradecanoate. A gap of 28 mass units occurs between m/e 171 and 199. This corresponds to cleavage on each side of the number 10 carbon. The mass spectrum of methyl 10-methyltetradecanoate is not in the mass spectral data base; however, all of the first five matches were fatty acid methyl esters.

Ethyl Palmitate--

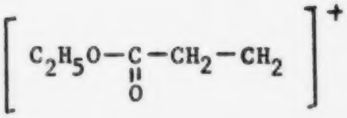
The computer unexpectedly matched two mass spectra with ethyl palmitate (ethyl hexadecanoate). To compound the confusion, one spectrum was from a peak that only occured in the methylated acid fraction of the Mill "A" lagoon effluent (Fig. 13-A) and the other spectrum occured in the neutral fraction only of the Mill "A" raw effluent (Fig. 13-B).

The base peak (m/e 88) and second largest fragment (m/e 101) are shifted 14 mass units higher than their counterparts in the mass spectra of saturated fatty acid methyl esters. These fragments would correspond to XII and XIV respectively.



$$\left[C_2H_5O-\underset{\substack{| \\ OH}}{C}=CH_2 \right]^+$$

XIII



$$\left[C_2H_5O-\underset{\substack{\| \\ O}}{C}-CH_2-CH_2 \right]^+$$

XIV

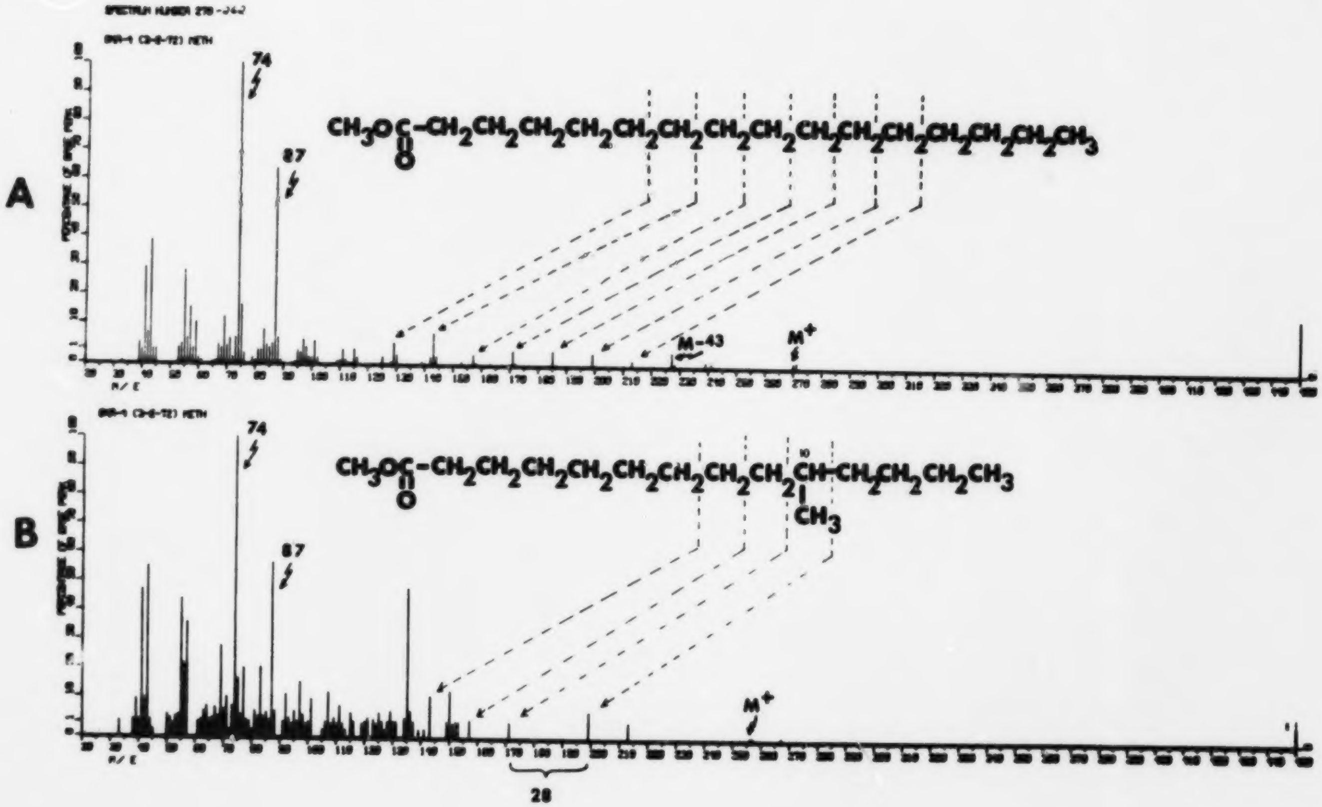


Figure 12 Mass spectra of (A) methyl palmitate and (B) tentatively identified methyl 10-methyltetradecanoate

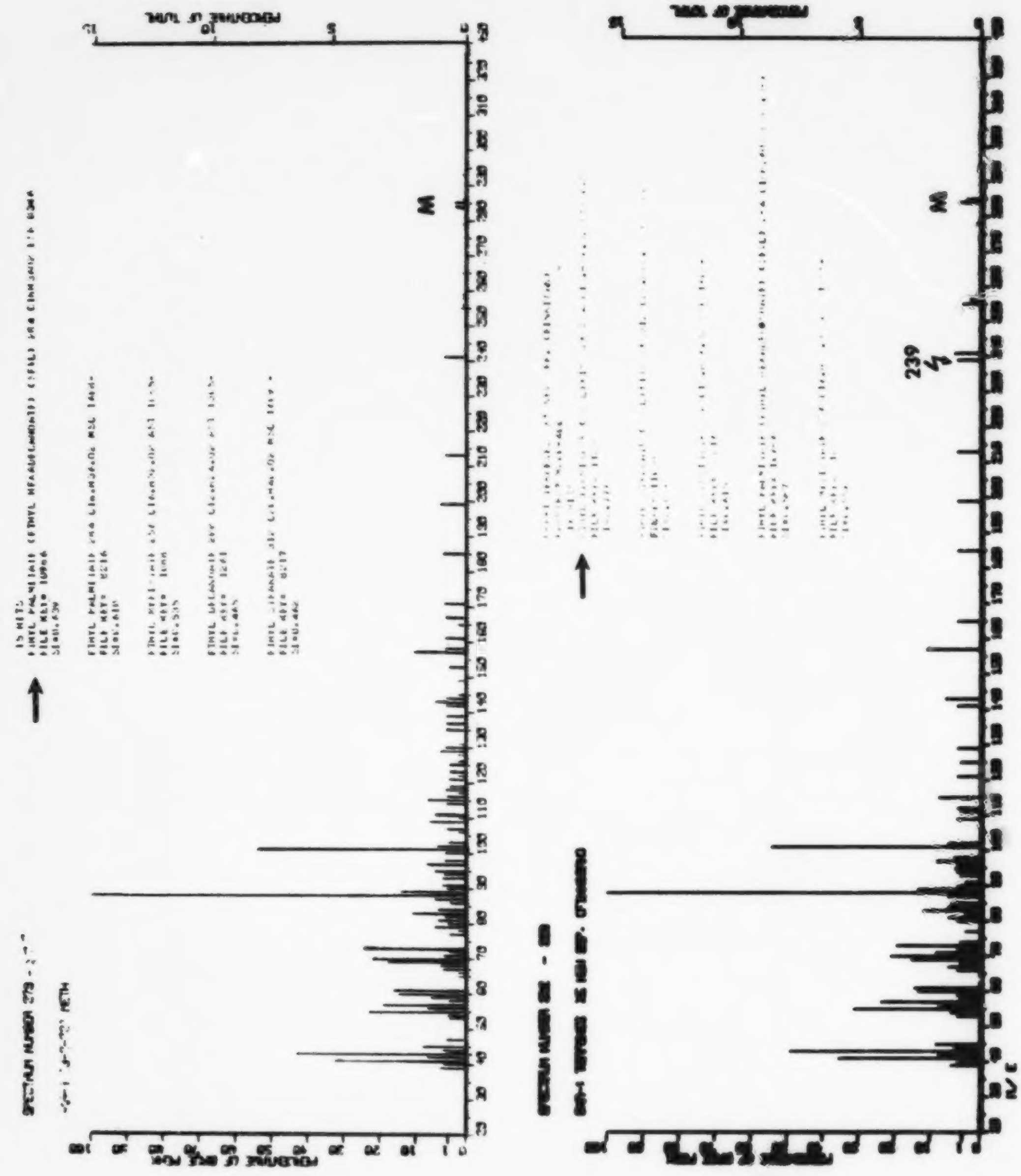
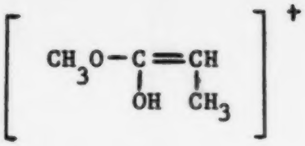


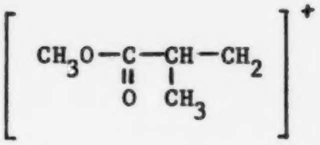
Figure 13 Mass spectra of ethyl palmitate found in (A) the methylated lagoon effluent extract of Mill "A" and (B) the neutral raw effluent extract of Mill "A"

However, they could also be rationalized as coming from structures XV and XVI. Corresponding to the compound methyl 2-methylhexadecanoate. The mass spectrum of the 2-methylhexadecanoate was not in the computer data bank.



$\left[CH_3O-\underset{OH}{C}=\underset{CH_3}{CH}\right]^+$

XV



$\left[CH_3O-\underset{O}{\underset{\|}{C}}-\underset{CH_3}{CH}-CH_2\right]^+$

XVI

An LMRGC generated for each sample using m/e 88 and m/e 101, indicated that no more compounds of this type were present in the extracts. The LMRGC of the methylated extract from Mill "A" lagoon wastewater is shown in Figure 14. Only one peak (shaded) contains both fragments.

The mass spectrum of methyl 2-methylhexadecanoate, (Fig. 15-A) closely resembles those of the sample spectra (Figs. 13-A and 13-B) with only some small variations. However, the methyl 2-methylhexadecanoate eluted from the GC much earlier than the unidentified compound in the samples.

The mass spectrum of a standard of ethyl palmitate (Fig. 15-B) also closely resembles the mass spectra of the sample peaks (Figs. 13-A and 13-B). However, methyl 2-methylhexadecanoate (Fig. 15-A) shows a fragment at m/e 253 (loss of OCH) and ethyl palmitate (Fig. 15-B) shows a fragment at m/e 239 (loss of OC_2H_5). Figure 13-B contains the m/e 239 fragment and no m/e 253 fragment. Figure 13-A does not show a m/e 239 fragment, but it shows no fragment at m/e 253 either. The m/e 239 peak was probably deleted during background subtraction since it is quite small.

The GC retention time of ethyl palmitate was identical to the peak in question. Spiking the samples with ethyl palmitate increased the size of this peak in the sample extracts, confirming the identity.

Unsaturated Fatty Acids--

Six unsaturated fatty acids were detected in the acid/phenol fraction of which three were identified (oleic, linoleic, and palmitelaidic acids). Most of the unsaturated fatty

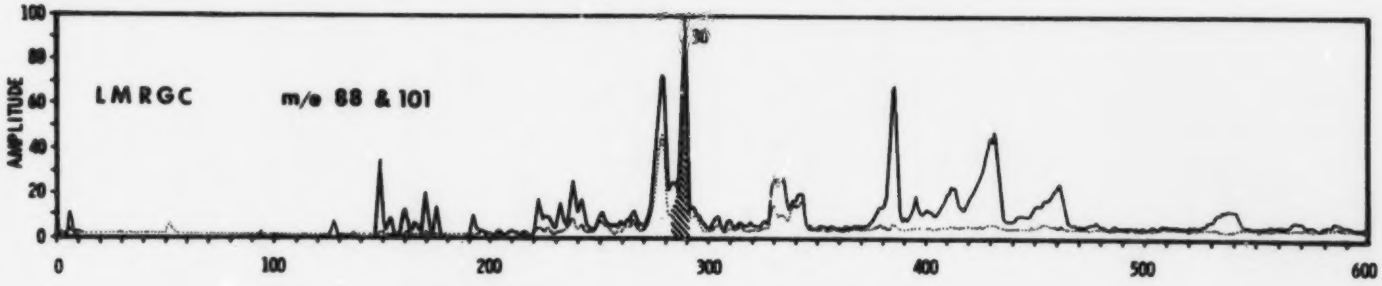


Figure 14 LMRGC of the methylated extract from Mill "A" lagoon effluent

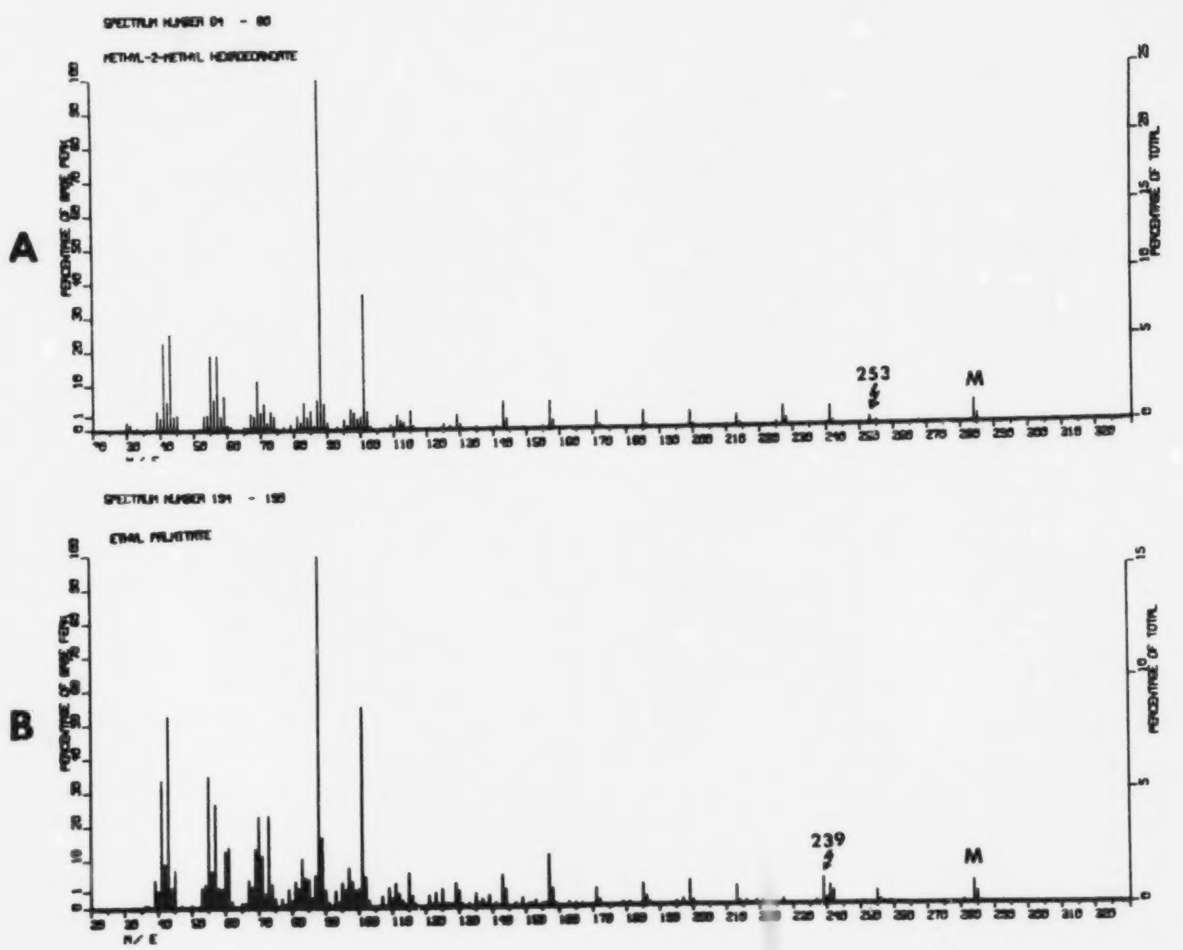


Figure 15 Mass spectra of (A) methyl 2-methylhexadecanoate and (B) ethyl palmitate (ethyl hexadecanoate)

acid concentration is contributed by oleic and linoleic acids.

Two additional unsaturated fatty acid methyl esters were found in the neutral extracts. These have been tentatively identified as a monounsaturated and a diunsaturated derivative of nonadecanoic acid (C-19). Their isomeric identities were not determined.

Fatty Acid Content of *Chlorella* Algae--

The increase in numbers of branched and odd-numbered carbon fatty acids in the lagoon effluents of both mills led us to consider that they could be metabolites of the aquatic life in the lagoons. Algae appeared to be a likely source of these compounds.

Accordingly, 1 liter of a culture of *Chlorella* algae common to the mill area was made basic with sodium hydroxide and extracted with chloroform to remove the neutrals. The alkaline solution was then made strongly acidic with hydrochloric acid and re-extracted to remove the acids. The acid extracts were evaporated almost to dryness in a Kuderna-Danish concentrator. A 0.10 ml aliquot of the concentrated extract was mixed with 0.03 ml MethElute and injected into a gas chromatograph for on-column methylation and analysis.

The gas chromatogram and the RGC are shown in Figures 16-A and 16-B respectively. The peak designated "R" in Figure 16-A is from the MethElute reagent. The peak designated with an asterisk in Figures 16-A and 16-B is di-n-butylphthalate, a contaminant. There was no evidence of branched fatty acids in this species of algae, and pentadecanoic acid was the only odd-numbered carbon fatty acid present. The acids identified and their relative abundances are listed in Table 3. This evidence does not invalidate the hypothesis that the odd-numbered and branched fatty acids originate as metabolites from aquatic life in the lagoons. Different species with different food sources can produce different compounds.

Resin Acids

A total of 13 different resin acids were found in the wastewaters of both mills in the 1972 samples; 8 were identified and confirmed and 5 were not identified. The resin acid content of the finished wastewaters were qualitatively similar for the two mills. Seven resin acids were common to

Table 3

FATTY ACIDS IN THE EXTRACT OF A *CHLORELLA* ALGAE CULTURE

Fatty Acid	Relative %	Found in Paper Mill Wastewater	Compound Name & Comments
12:0	0.4	No	Lauric acid
14:0	1.6	Yes	Myristic acid
15:0	0.7	Yes	Pentadecanoic acid
16:0	19.0	Yes	Palmitic acid
16:2	17.0	No	cis or trans not determined
16:3	23.2	No	cis or trans not determined
18:0	4.9	Yes	Stearic acid
18:1	6.2	Yes	Oleic or elaidic acid
18:2	13.8	Yes	Linoleic or linolelaidic acid
18:3	13.2	Sometimes	Linolenic or gamma-linolenic acid

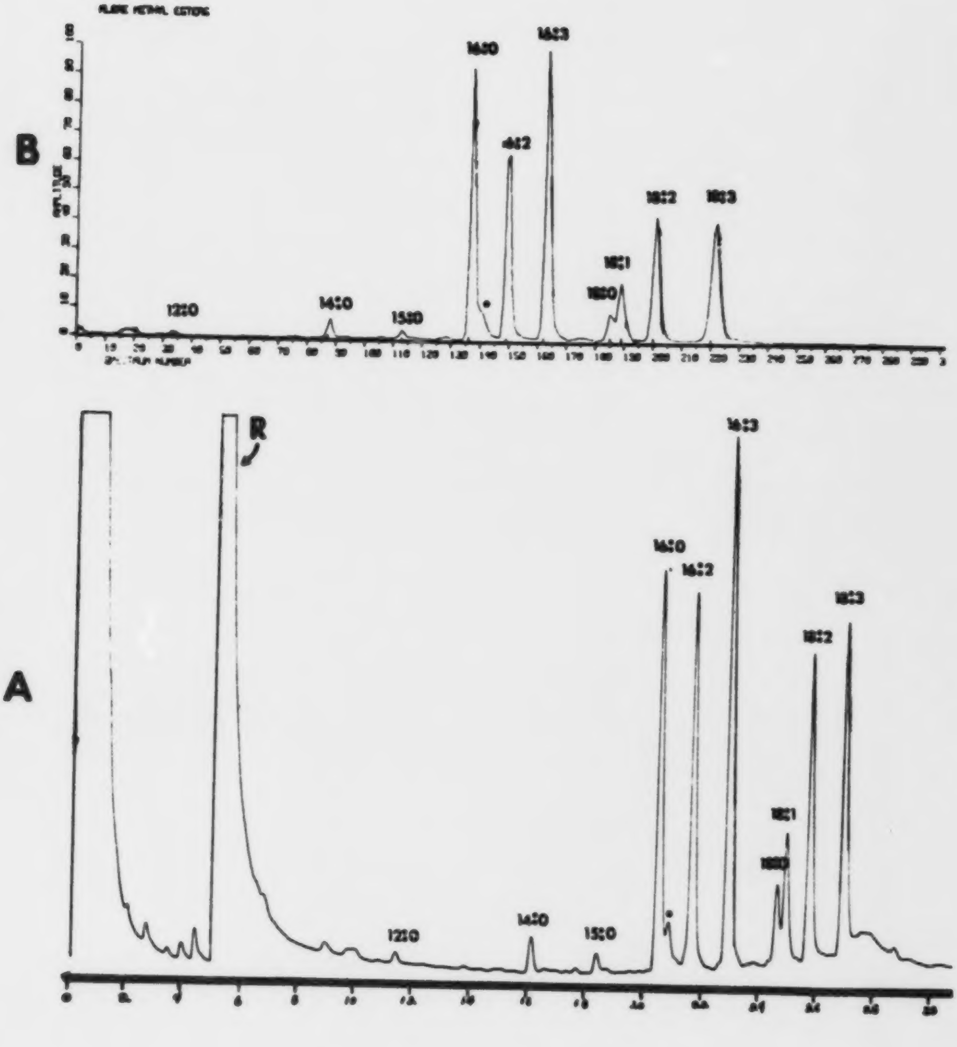


Figure 16 (A) Gas chromatogram and (B) RGC of fatty acid methyl esters from chlorella algae

the raw wastewater extracts of both mills and six of these were found in both finished wastewater extracts. Differences were primarily found among the small, unidentified resin acid GC peaks.

Significance of Resin Acids--

Resin acids have long been known to be toxic to various degrees to aquatic life. In addition, resin acid salts or "soaps" are responsible for much of the foaming in kraft mill effluents. The foam causes additional expense in the treatment of these effluents because defoamers must be added. If the foam is discharged to the receiving waters it can float long distances before it is dispersed or degraded.[3]

Dr. I. H. Rogers[29] has recently reported that bioassays using sockeye salmon have shown the lethal effects of a mixture of resin acids isolated from Douglas fir oleoresin to occur at concentrations as low as 2.0 mg/l. Others have found resin acid soaps to be toxic to minnows at 1.0 mg/l[30] or to the water flea, Daphnia pulex,, at 2.0 mg/l[31]. Resin acids have been reported toxic to various fish at concentrations of 1 mg/l[32] to 5 mg/l.[33]

Leach and Thakore[34] recently reported that 80% of the toxicity of unbleached white water (the major waste stream from the pulping operation of a kraft mill) to juvenile coho salmon was caused by the sodium salts of three resin acid soaps: sodium isopimarate (55%), sodium abietate (22%), and sodium dehydroabietate (5%). The acute toxicity of free resin acids was much less than that of the sodium salts. The pimaric-type acids were also found to be more toxic than the abietic-type resin acids. The remaining toxicity (18%) was contributed by sodium salts of the unsaturated fatty acids: sodium palmitolate, sodium oleate, sodium linoleate, and sodium linolenate.

Identification of Resin Acids--

In addition to the isopimaric, abietic, dehydroabietic and sandaracopimaric acids mentioned by Leach and Thakore[34], we found pimaric, 6,8,11,13-abietatraen-18-oic, neoabietic, 13-abieten-18-oic and several unidentified resin acids. To our knowledge neoabietic, 13-abieten-18-oic, and 6,8,11,13-abietatraen-18-oic acids have not been reported before in kraft pulp mill wastewaters. All of these resin acids (as their methyl esters) were initially identified by comparison of their mass spectra with published mass spectra.[35] They were all confirmed by comparison of gas chromatographic retention times and GC-mass spectra from our instrument with standards obtained from Dr. Duane F. Zinkel, U.S.D.A. Forest Products Laboratory, Madison, Wisconsin.

The mass spectra and fragmentation patterns of these resin acid methyl esters are covered in the literature[36] and will not be discussed here. The plotted spectra, however are shown in Figures 17-20 because some of them have ion abundances different from those reported in literature references.[35] These differences are primarily the incorporation of masses between m/e 41 and 70 in our plots, and may be due to the GC mode of introduction (versus direct insertion probe[35] [36]) with our samples and to our using a quadrupole mass spectrometer instead of a magnetic instrument.

The unidentified resin acid methyl esters may be decomposition products or rearranged molecular structures from a parent resin acid. The mass spectra of some may also be contaminated with mass spectra of other unresolved compounds or may be resin acids not previously identified and studied.

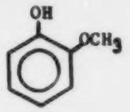
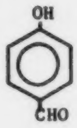
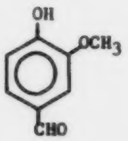
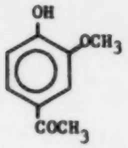
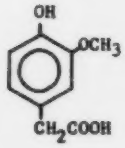
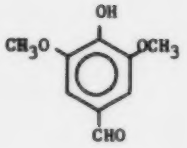
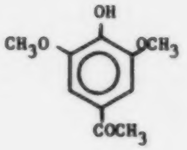
Phenols

Eleven different phenols were identified in the kraft mill wastewaters (Table 2). Guaiacol (identified as its methyl ether, veratrole) varied in concentration from 0.5 to 2.7 mg/l in the raw effluents and was very effectively reduced in concentration by both treatments. Vanillin (identified as its methyl ether, veratraldehyde) varied in concentration from 0.8 to 2.1 mg/l in the raw effluents but was not as effectively reduced in concentration as guaiacol. The other nine phenols occured in lesser concentrations and varied in simplicity from phenol to acetosyringone (3,5-dimethoxy-4-hydroxyacetophenone).

Based upon data obtained in 1974, some correlation can be made between the percent removal of the phenols in these waste treatment systems and the complexity or degree of substitution of the phenol molecules. Table 4 lists the removal data for these phenols and shows their structures. The analytical data for the calculations were obtained from 3-day composite samples, quantitated with the aid of a Perkin-Elmer PEP-1 computer system. (The details of these analyses are described in a later section.) The phenols are generally more resistant to treatment as the complexity of the molecule increases. This is especially true with Mill "A", which has only biological treatment, with the single

Table 4

PERCENT REMOVAL OF PHENOLS VERSUS THEIR STRUCTURAL COMPLEXITY

Parent Compound	Identified As	Structure	% Removed Mill "A"	 Interstate
Guaiacol	Veratrole		96%	98%
p-Hydroxybenzaldehyde	p-Methoxybenzaldehyde		21%	75%
Vanillin	Veratraldehyde		65%	93%
Acetovanillone	Acetoveratrone	 OH OCH$_3$ COCH$_3$	77%	90%
Homovanillic Acid	Methyl Homovanillate	 OH OCH$_3$ CH$_2$COOH	50%	?
Syringaldehyde	3,4,5-Trimethoxy-benzaldehyde	 OH CH$_3$O OCH$_3$ CHO	62%	--
Acetosyringone	3,4,5-Trimethoxy-acetophenone	 OH CH$_3$O OCH$_3$ COCH$_3$	57%	67%

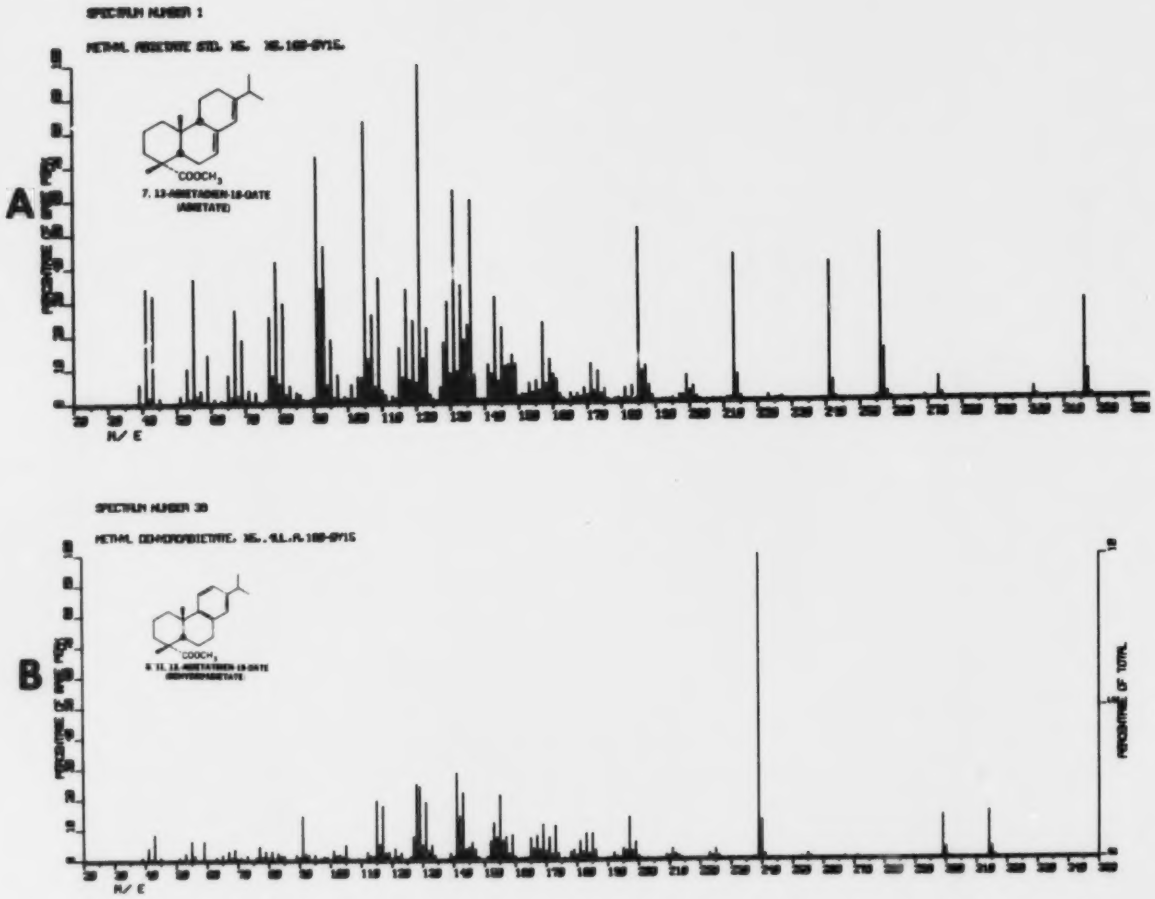


Figure 17 Mass spectrum of (A) methyl abietate and (B) methyl dehydroabietate

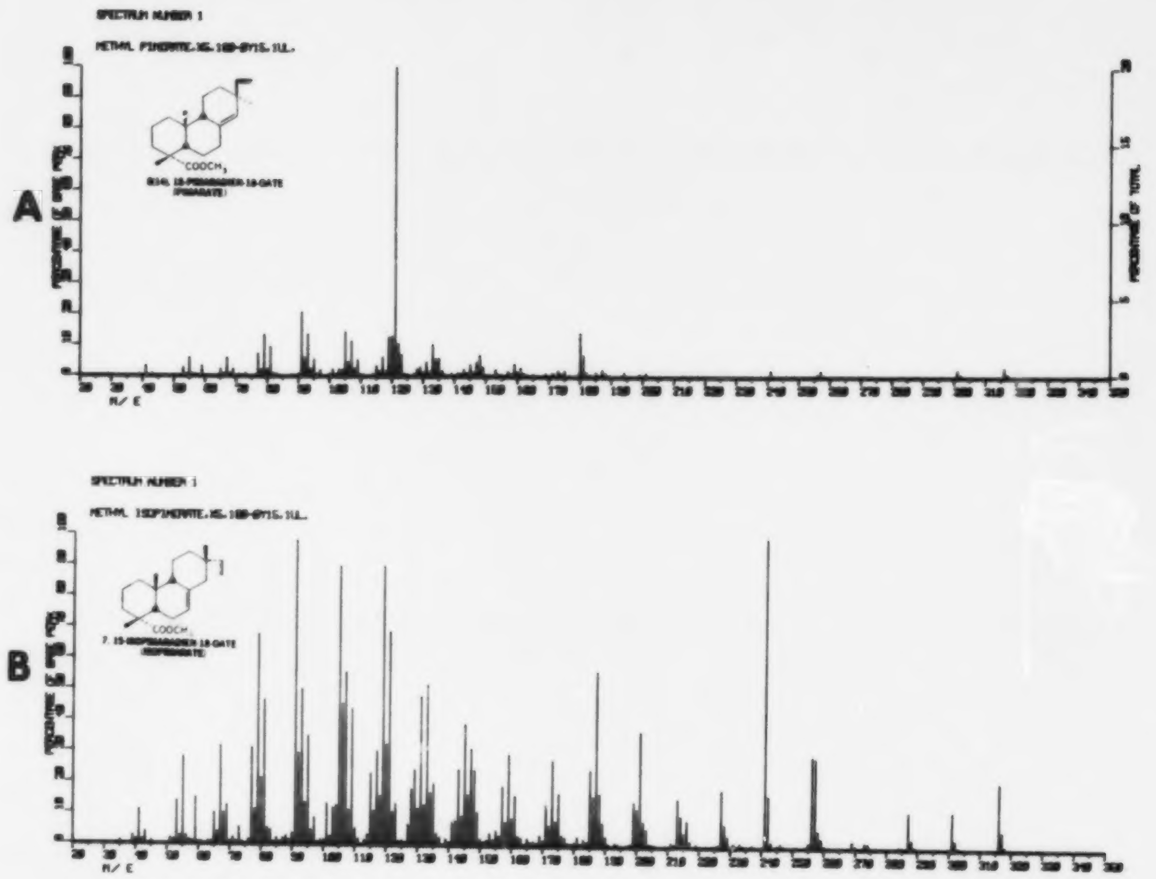


Figure 18 Mass spectrum of (A) methyl pimarate and (B) methyl isopimarate

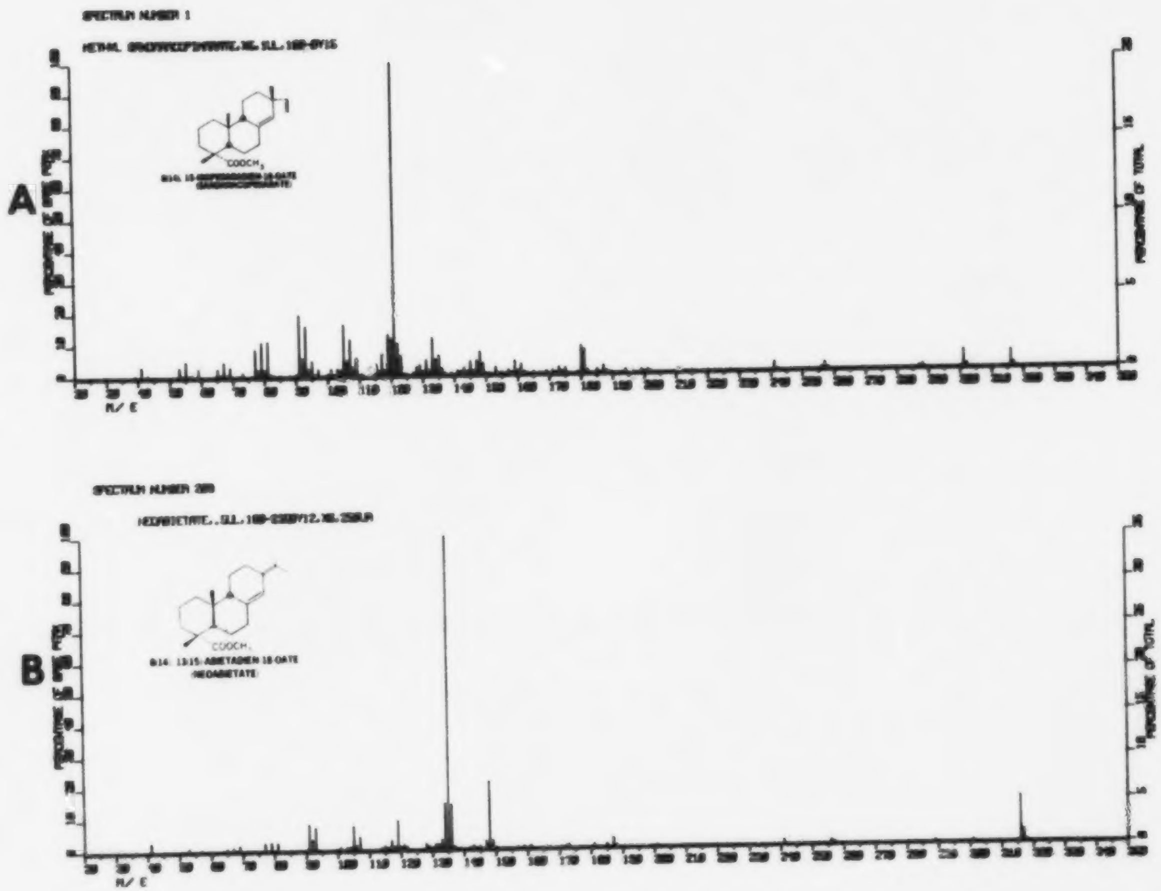


Figure 19 Mass spectrum of (A) methyl sandaracopimarate and (B) methyl neoabietate

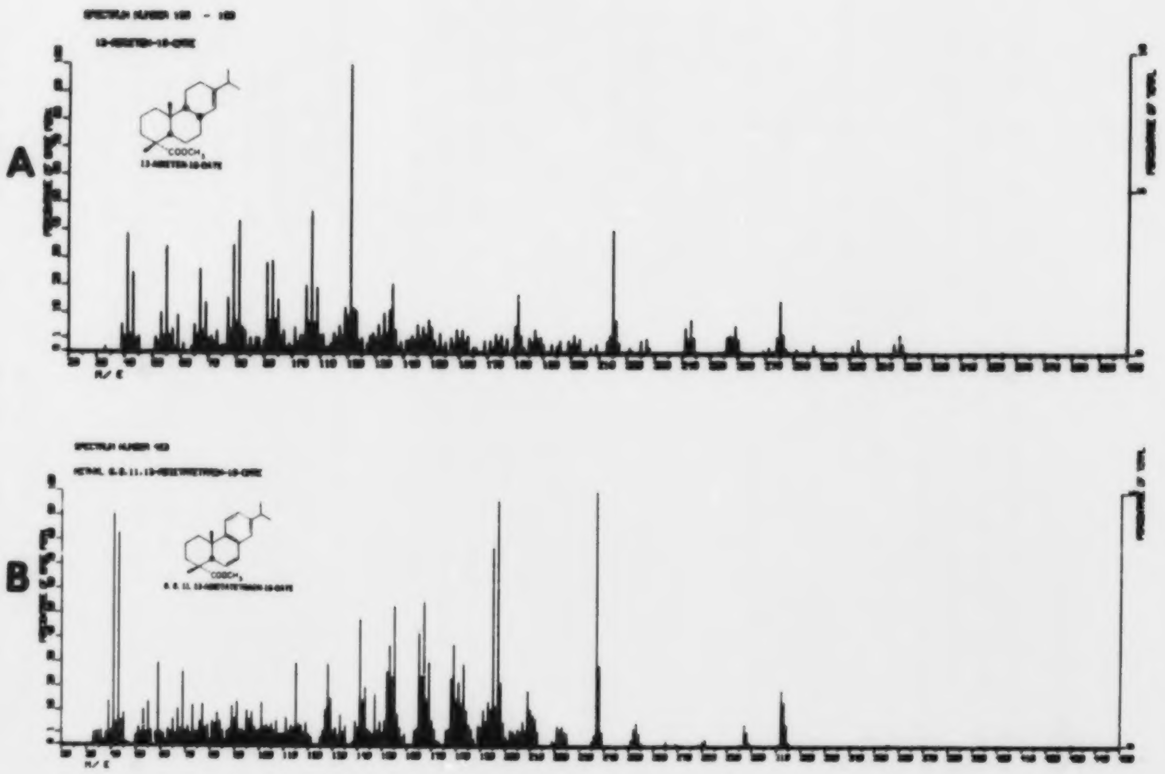


Figure 20 Mass spectrum of (A) methyl 13-abieten-18-oate and (B) methyl 6,8,11,13-abietatetraen-18-oate

exception of p-hydroxybenzaldehyde, which may be produced in the aerated lagoons by biological degradation of lignin or vanillin. Most of these phenols are probably involved in complex equilibria, and their concentrations depend upon their initial concentrations in the wastewater, their rates of degradation in the lagoons, and their rates of formation from degradation of lignin and other molecules in the lagoons.

Significance of Phenols--

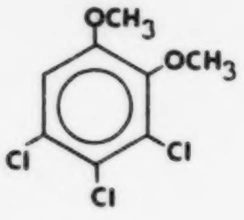
Phenols have long been associated with kraft paper mill wastewaters and many studies of their effects have been made. Chopin[37] reported that phenolic compounds extracted from various pulping processes are slightly toxic but much less so than phenol or cresols. Concentrations of phenol that have been reported as lethal or damaging to fish range from 0.079 to 1900 mg/l but the most reliable information relating to pure phenol under carefully standardized conditions indicates that the 24-, 48- and 96-hour TLm concentrations are in the general range of 10-20 mg/l at 20°C.[38]

Probably a greater problem caused by the phenolic constituents of kraft mill wastewaters is the impairment of the flavor of fish, shrimp, and other edible aquatic life. A table in a recent report[39] lists the highest concentrations of various organics in water that will not impair fish flavor. Values for phenolics range from 0.005 mg/l for cresol to 5.6 mg/l for phenol. The only compound in the table that was found in the treated mill wastewaters is guaiacol. The highest concentration of guaiacol that would not impair the flavor of resident fish was listed as 0.1 mg/l.

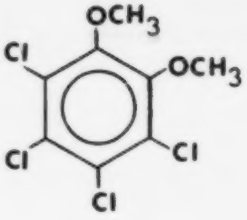
An earlier investigation[40] showed that the flavor of cooked coho salmon was impaired after exposure of the fish to untreated kraft pulp mill effluent for 72-96 hours at concentrations of 1-2% or more by volume. No flavor impairment was noted when these fish were exposed to 2-9% by volume of biologically-treated effluent. These results are in agreement with our findings that, in general, the phenolic constituents of kraft paper mill wastewaters are reduced in concentration from 50 to 98% by biological treatment.

In some kraft paper mills, the pulp is bleached with chlorine. Analyses of several wastewater extracts from bleached kraft mills in collaboration with Dr. I. H. Rogers, Environment Canada, Pacific Environmental Institute, West

Vancouver, B.C., have led to the identification of 3,4,5-trichloroveratrole XVII and tetrachloroveratrole XVIII .[41] Since the samples were methylated prior to analysis, the parent compounds were chlorinated guaiacols.



XVII



XVIII

Phenols are known to be highly susceptable to chlorination under the conditions employed in the bleaching operations of most mills. Guaiacol was subjected to chlorination under conditions approximating those used in the mill from which the samples were taken and a series of chlorinated isomers resulted. The only trichloroisomer found was 3,4,5-trichloroguaiacol, identified and confirmed as its methyl derivative (XV) by GC-MS, NMR, and GC-IR. The only possible tetrachloro isomer (XVI) was also found. The results of these and other related analyses are fully described in a separate report.[41]

Generally, chlorinated phenols are more toxic than the parent compounds and the concentrations at which flavor in fish is impaired or at which taste and odor can be detected in drinking water is much lower for the chlorinated compounds. For example, the highest concentration of 2,4-dichlorophenol that does not impair fish flavor is 1/560,000 that of phenol for trout and 1/56,000 that of phenol for bass.[39] The upper limit for isomeric 2,3-dichlorophenol for trout is 1/175 that of phenol for trout. A great deal of research must be done with the phenols identified to determine their chlorinated derivatives produced in mill bleaching operations, and the effects of these chlorinated phenolics on aquatic life and in drinking water.

CHEMICAL PROFILES OF ACIDS AND PHENOLS

A "chemical profile" is a vertical display of the gas chromatograms of samples taken at consecutive points in a waste treatment system. The compounds present and their relative concentrations are therefore shown as a function of the treatment.

This display allows one to see at a glance the effectiveness (or lack of it) of each step in the treatment process with respect to an individual compound, to a class of compounds, or to all chromatographable compounds in the wastewater, whether they are identified or not.

This information in conjunction with the traditional collective pollution parameters (e.g., BOD and TOC) provides a much better understanding and evaluation of the effluent composition and its possible environmental effects than the chemical profiles or collective parameters alone.

Figure 21 shows the chemical profile of the acids and phenols from the wastewater grab samples of Mill "A". Figure 22 shows the chemical profile of the acids and phenols from the grab samples of the Interstate mill at Riceboro, Georgia. The peak numbers correspond to the compounds listed in Table 2.

The compositions of the raw effluents from the two mills were similar. The treated effluent samples from the two mills exhibited more differences. In general, most compounds decreased significantly in concentration as the wastewater passed through the treatment system.

One exception appeared to be the resin acid content. Some of the peaks appeared to increase in concentration. This apparently was the result of large variances in the concentration of the organics in the mill wastewaters with time and our failure to have precisely sampled the "slug" of effluent we tried to follow through the treatment systems. Another factor could be that the biological parts of one or both of these systems was not working at usual efficiency when the samples were taken. In any event, the quantitation of the compounds in 1972 samples was not accurate. The major effort was directed at the identification of these compounds and their verification with standards.

REPEAT ANALYSES OF THE ACIDS AND PHENOLS

Additional samples from both mills were obtained in January 1974--almost 2 years after the previous samples were taken. The analyses were repeated for two reasons:

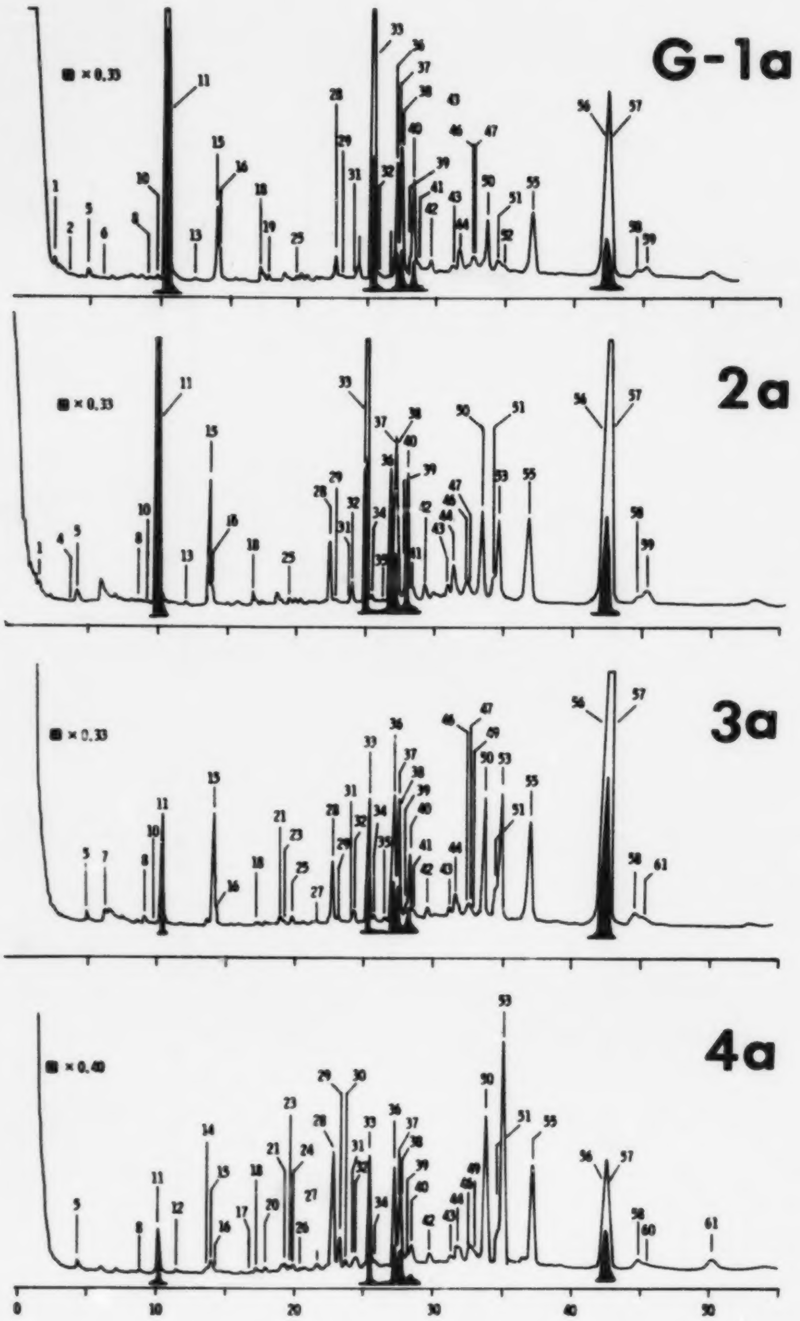


Figure 21 Chemical profile of acids and phenols from Mill "A"; sample points 1-4.

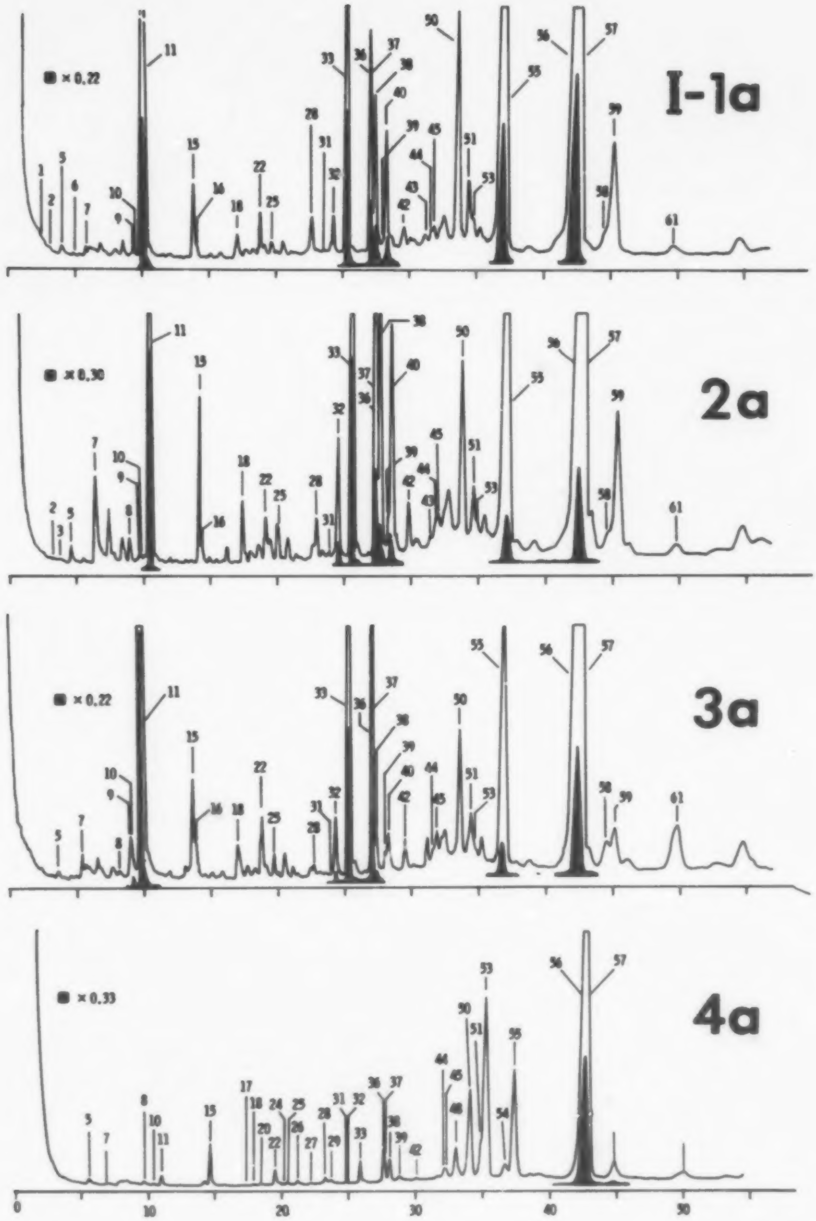


Figure 22 Chemical profile of acids and phenols from the Interstate mill at Riceboro, Georgia; sample points 1-4

- to ascertain whether the same compounds were still present and, if so, if they were present in approximately the same concentrations.

- to obtain reliable, representative, quantitative data on the concentrations of these compounds.

The first objective is important if the information obtained from these studies is to be of general use. A radical change in the wastewater composition with time would require each analysis to be a research project--an impractical situation. However, if the composition of the wastewaters is essentially the same over a two-year time span, then the assumption that the wastewater composition will remain the same is basically valid, unless major changes in raw materials, production practices, or treatment processes occur.

The second objective is important if the information obtained from these studies is to be used to assess the effectiveness of the treatments towards reducing the concentration of a class of chemicals or even of a specific compound.

Sampling

Samples from both mills were collected by automatic sampling devices at the mills. All samples were collected during the same week. Samples of the raw wastewaters and of the outfalls, collected over 24-hour periods, were frozen each day. A 3-day composite composed of equal volumes from the Monday, Tuesday, and Wednesday raw wastewater samples of Mill "A" was prepared. A second 3-day composite was composed of equal volumes from the Wednesday, Thursday and Friday treated wastewater samples of Mill "A". This provided samples of a slug of wastewater, with a 3-day averaged composition, both before and after it had passed through the 2.5-day treatment system.

Three-day composites of equal volumes from the Monday, Tuesday and Wednesday raw wastewater and of the treated wastewater samples of the Interstate Mill at Riceboro were prepared. Because the stabilization pond retention time is at least 3 months, no attempt was made to obtain samples of the same slug of this wastewater.

Samples were not taken at intermediate points in the treatment system of either mill because automatic sampling devices were not available there and because analyses time was limited.

Method of Analysis

A one-liter aliquot of each of the four composite samples was taken and was prepared in essentially the same manner as were the 1972 samples:

- After the pH was raised to 10.5 with 50% sodium hydroxide, the terpenes and neutral compounds were extracted with chloroform.
- The aqueous sample was divided into two 500-ml portions and each portion was methylated using dimethyl sulfate.
- After extraction of the methylated portions with chloroform, the extracts of each sample were combined and concentrated to 1.0 ml. By utilizing two separate methylation reactions for each sample, some averaging of either a low- or a high-yield methylation reaction is gained.

The samples were spiked with acenaphthene as an internal standard and chromatographed on a 50-ft SCOT column packed with carbowax 20M/TPA. Retention time and peak area data were collected on a Perkin Elmer PEP-1 computer interfaced with the gas chromatograph. Computer analysis of these data allowed identification and quantitation of the compounds based on information gained from the 1972 samples. PEP-1 computer identifications were verified by obtaining mass spectra of the compounds in these samples and comparing them with the mass spectral data of the identified compounds from the 1972 samples.

COMPUTER ASSISTED GAS CHROMATOGRAPHIC ANALYSES

Based on the compound identifications from the 1972 analyses, a computer-assisted gas chromatographic analysis was used to analyze the 1974 wastewater samples. GC-MS was used to verify compound identifications and showed that, once identified, computer-assisted GC analysis was sufficient for additional analyses.

Response Factors of Model Compounds

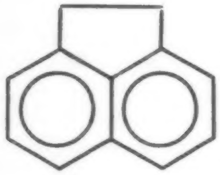
A standard solution consisting of 5.75 mg/l veratrole, 1.12 mg/l acenaphthene, 2.82 mg/l methyl palmitate, and 8.96 mg/l methyl dehydroabietate was prepared and chromatographed using the PEP-1 computer system. From the known concentrations and the measured peak areas, response factors were calculated relative to that of acenaphthene, which, as the internal standard, is assigned a value of 1.00. The response factors were as follows:

Acenaphthene	1.00	(internal standard)
Veratrole	1.49	(phenols)
Methyl palmitate	2.05	(fatty acid methyl esters)
Methyl dehydroabietate	2.32	(resin acid methyl esters)

Assuming all compounds in the same chemical class have the same response factor, the above factors were assigned to the various phenols and fatty and resin acid methyl esters so the computer could calculate the concentrations of these compounds in the samples.

Analyses of the 1974 Samples

A 300-μl aliquot of each extract was spiked with 30 μl of a standard solution (11 mg/ml) of acenaphthene (XIX), bringing its concentration, in the extract to 1.0 mg/ml. Since extracts of 1 liter of each sample had been concentrated to 1.0 mg/l, the concentrations of the various components (in mg/l) could be obtained by multiplying the appropriate peak area by the corresponding response factor and comparing the product with the acenaphthene peak area.



XIX

The Perkin-Elmer Multiple Reference Peak computer program was used for determining relative retention times instead of the Single Reference Peak program because the time required for each chromatogram approaches 50 minutes. Peaks corresponding to veratrole, veratraldehyde and methyl dehydroabietate were chosen as retention time reference peaks.

An example of the computer program used to analyze the raw effluent extract from the Interstate mill at Riceboro is shown in Figure 23. The raw effluent reduced data, showing peak areas normalized to the sum of all the peak areas in the run, is shown in Figure 24. The product of the computer analysis of the data in Figure 24 using the program in Figure 23 is shown in Figure 25. The peak number designations, the notation of the presence or absence of the compound in the corresponding 1972 sample, and the total from the sum of all the concentration values in Figure 25, were manually added to the data printed out by the computer.

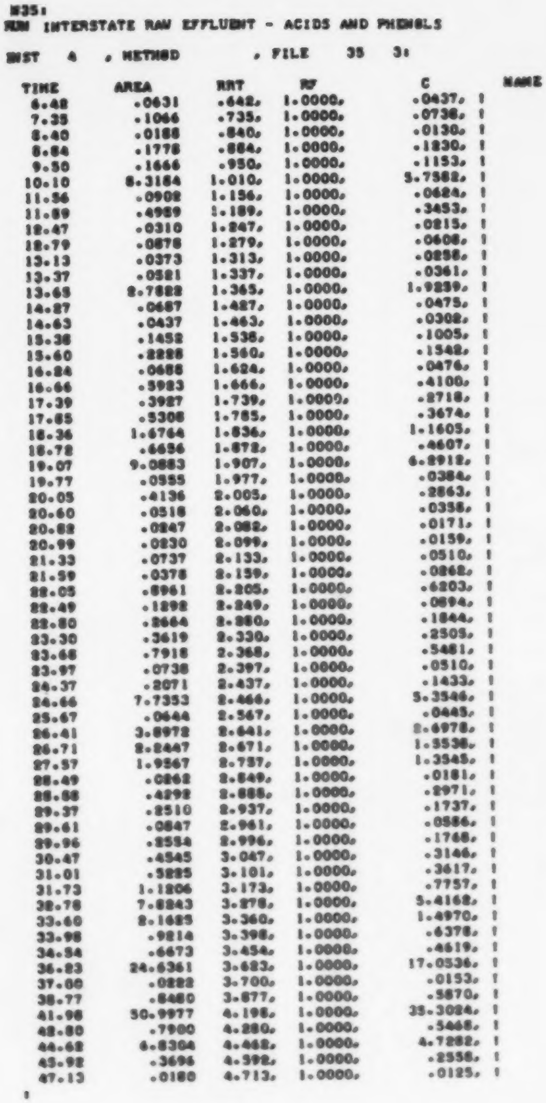
The gas chromatogram of the extract from the Interstate mill's raw wastewater is shown in Figure 26-A. Peak number designations match those in Figure 25 and are consistent with identifications made with the 1972 samples. The letter "A" designates the acenaphthene spike used for the internal standard. The letter "M" designates methyl margarate, which was not included in the list of identifications in the 1972 samples because the peak resolution was not as good and the confirmation was doubtful.

The gas chromatogram and computer analysis of the extract from Interstate's treated effluent (sample point #4 in Figure 3) are shown in Figures 27 and 26-B respectively. A comparison of either Figures 25 and 27 or of 26-A and 26-B shows significant reductions in the concentrations of these compounds after treatment.

Similar comparisons can be made from the computer analyses of Mill "A" raw effluent and treated effluent extracts (Figs. 28 and 29) or from the gas chromatograms of these same extracts (Figs. 30-A and 30-B). Analyses of the raw effluent were run in duplicate (Fig. 28) to determine the range of agreement found using the PEP-1 system. Twenty of the values were within a 1% to 10% range of agreement with each other. Three of the values were greater than the 10% range of agreement (35%, 38% and 53%).

```
P50:
RUN   INTERSTATE RAW EFFLUENT-ACIDS AND PHENØLS:

INST   1    , METHØD   50   , FILE    40   3:

STD CØNC        1.0000:

TIMES   48.00,    9.00,   23.55,   26.55,   30.20,   33.75,

THRESHØLDS    16,    32,

UNK/AIR   1.0000,     .00,

TØL   .140,    .010,    5.0,

REF
   1.000,    9.50,   10.50,   20.00,

   2.450,   24.00,   25.00,   30.00,

   4.100,   40.50,   42.00,   48.00, :

STD NAME    ACENAPHTHENE:

     RRT        RF             C          NAME
   1.000,     1.4900,           .0000, VERATRØLE:
   1.350,     2.0500,           .0000, DIMETHYLSULFØNE:
   1.650,     1.4900,           .0000, METHØXYBENZALDEHYDE:
   1.760,     1.0000,           .0000,    :
   1.770,     1.0000,           .0000,    :
   1.817,     1.0000,           .0000,    :
   1.850,     1.4900,           .0000, ARØMATIC MW 182:
   1.890,     1.0000,          1.0000, ACENAPHTHENE:
   1.980,     1.0000,           .0000,    :
   2.190,     2.0500,           .0000, PALMITATE:
   2.260,     1.0000,           .0000,    :
   2.330,     2.0500,           .0000, ANTEISØMARGARATE:
   2.350,     1.4900,           .0000, ME HØMØVANILLATE:
   2.430,     2.0500,           .0000, MARGARATE:
   2.450,     1.4900,           .0000, VERATRALDEHYDE:
   2.620,     1.4900,           .0000, VERATRØNE:
   2.650,     2.0500,           .0000, STEARATE AND ØLEATE:
   2.750,     2.0500,           .0000, MØSTLY LINØLEATE:
   2.870,     1.4900,           .0000, 3,4,5-TMA:
   2.930,     1.0000,           .0000,    :
   2.990,     2.0500,           .0000, ARACHIDATE:
   3.030,     2.3200,           .0000, ME RESIN ACID:
   3.080,     2.3200,           .0000, ME RESIN ACID:
   3.145,     2.3200,           .0000, ME RESIN ACID MW 314:
   3.240,     2.3200,           .0000, PIMERATE:
   3.360,     2.3200,           .0000, SANDARACØPIMERATE:
   3.400,     2.3200,           .0000, 13-ABIETEN-18-ØATE:
   3.410,     1.0000,           .0000,    :
   3.560,     2.3200,           .0000, ISØPIMERATE:
   3.880,     1.0000,           .0000,    :
   4.100,     2.3200,           .0000, AB- AND DEHYDRØAB-:
   4.170,     1.0000,           .0000,    :
   4.350,     2.3200,           .0000, 6,8,11,13-AB, NEØAB-:
   4.600,     2.0500,           .0000, LIGNØCERATE:
```

Figure 23 Print-out of the computer program used to analyze the raw effluent extract from the Interstate mill at Riceboro



```
#35:
RUN INTERSTATE RAW EFFLUENT - ACIDS AND PHENOLS

INST   4   , METHOD          , FILE    35   3:

  TIME        AREA        RRT      RF                 C            NAME
  6.42        .0631      .642,  1.0000,            .0437,  !
  7.35        .1066      .735,  1.0000,            .0738,  !
  8.40        .0188      .840,  1.0000,            .0130,  !
  8.84        .1778      .884,  1.0000,            .1230,  !
  9.50        .1666      .950,  1.0000,            .1153,  !
 10.10       8.3184     1.010,  1.0000,           5.7582,  !
 11.56        .0902     1.156,  1.0000,            .0624,  !
 11.89        .4989     1.189,  1.0000,            .3453,  !
 12.47        .0310     1.247,  1.0000,            .0215,  !
 12.79        .0878     1.279,  1.0000,            .0608,  !
 13.13        .0373     1.313,  1.0000,            .0258,  !
 13.37        .0521     1.337,  1.0000,            .0361,  !
 13.65       2.7822     1.365,  1.0000,           1.9239,  !
 14.27        .0687     1.427,  1.0000,            .0475,  !
 14.63        .0437     1.463,  1.0000,            .0302,  !
 15.38        .1452     1.538,  1.0000,            .1005,  !
 15.60        .2228     1.560,  1.0000,            .1542,  !
 16.24        .0688     1.624,  1.0000,            .0476,  !
 16.66        .5923     1.666,  1.0000,            .4100,  !
 17.39        .3927     1.739,  1.0000,            .2718,  !
 17.85        .5308     1.785,  1.0000,            .3674,  !
 18.36       1.6764     1.836,  1.0000,           1.1605,  !
 18.72        .6656     1.872,  1.0000,            .4607,  !
 19.07       9.0883     1.907,  1.0000,           6.2912,  !
 19.77        .0555     1.977,  1.0000,            .0384,  !
 20.05        .4136     2.005,  1.0000,            .2863,  !
 20.60        .0518     2.060,  1.0000,            .0358,  !
 20.82        .0247     2.082,  1.0000,            .0171,  !
 20.99        .0230     2.099,  1.0000,            .0159,  !
 21.33        .0737     2.133,  1.0000,            .0510,  !
 21.59        .0378     2.159,  1.0000,            .0262,  !
 22.05        .8961     2.205,  1.0000,            .6203,  !
 22.49        .1292     2.249,  1.0000,            .0894,  !
 22.80        .2664     2.280,  1.0000,            .1844,  !
 23.30        .3619     2.330,  1.0000,            .2505,  !
 23.68        .7918     2.368,  1.0000,            .5481,  !
 23.97        .0738     2.397,  1.0000,            .0510,  !
 24.37        .2071     2.437,  1.0000,            .1433,  !
 24.66       7.7353     2.466,  1.0000,           5.3546,  !
 25.67        .0644     2.567,  1.0000,            .0445,  !
 26.41       3.8972     2.641,  1.0000,           2.6978,  !
 26.71       2.2447     2.671,  1.0000,           1.5538,  !
 27.57       1.9567     2.757,  1.0000,           1.3545,  !
 28.49        .0262     2.849,  1.0000,            .0181,  !
 28.88        .4298     2.888,  1.0000,            .2971,  !
 29.37        .2510     2.937,  1.0000,            .1737,  !
 29.61        .0847     2.961,  1.0000,            .0586,  !
 29.96        .2554     2.996,  1.0000,            .1768,  !
 30.47        .4545     3.047,  1.0000,            .3146,  !
 31.01        .5225     3.101,  1.0000,            .3617,  !
 31.73       1.1206     3.173,  1.0000,            .7757,  !
 32.78       7.8243     3.278,  1.0000,           5.4162,  !
 33.60       2.1625     3.360,  1.0000,           1.4970,  !
 33.98        .9214     3.398,  1.0000,            .6378,  !
 34.54        .6673     3.454,  1.0000,            .4619,  !
 36.23      24.6361     3.623,  1.0000,          17.0536,  !
 37.00        .0222     3.700,  1.0000,            .0153,  !
 38.77        .8480     3.877,  1.0000,            .5870,  !
 41.98      50.9977     4.198,  1.0000,          35.3024,  !
 42.80        .7900     4.280,  1.0000,            .5468,  !
 44.62       6.8304     4.462,  1.0000,           4.7282,  !
 45.92        .3696     4.592,  1.0000,            .2558,  !
 47.13        .0180     4.713,  1.0000,            .0125,  !
 !
```

Figure 24 Computer print-out of the normalized peak areas from the chromatogram of the raw effluent extract from the Interstate mill at Riceboro

```
RUN          INTERSTATE RAW EFFLUENT-ACIDS AND PHENOLS

INST   4   , METHOD   50   , FILE    35    3:

  TIME        AREA       RRT       RF              C          NAME                          PEAK      IN '72
  10.10      6.3184    1.000,   1.4900,        1.3637,  VERATROLE:                          11         YES
  11.89       .4989    1.177,   1.0000,         .0548,  !                                   --         --
  13.65      2.7822    1.351,   2.0500,         .6275,  DIMETHYLSULFONE:                    15         YES
  15.60       .2228    1.544,   1.0000,         .0245,  !                                   --         --
  16.66       .5923    1.649,   1.4900,         .0971,  METHOXYBENZALDEHYDE:                18         YES
  17.39       .3927    1.721,   1.0000,         .0432,    :                                 --         --
  17.85       .5308    1.767,   1.0000,         .0584,    :                                 --         --
  18.36      1.6764    1.817,   1.0000,         .1844,    :                                 --         --
  18.72       .6656    1.853,   1.4900,         .1091,  AROMATIC MW 182:                    --          NO
  19.07      9.0883    1.888,   1.0000,        1.0000,  ACENAPHTHENE:                       A          --
  20.05       .4136    1.985,   1.0000,         .0455,    :                                 --         --
  22.05       .8961    2.186,   2.0500,         .2021,  PALMITATE:                          26         YES
  22.80       .2664    2.262,   1.0000,         .0293,    :                                 --         --
  23.30       .3619    2.312,   2.0500,         .0816,  ANTEISOMARGARATE:                   31         YES
  23.68       .7918    2.351,   1.4900,         .1298,  ME HOMOVANILLATE:                   32         YES
  24.37       .2071    2.420,   2.0500,         .0467,  MARGARATE:                          M           NO
  24.66      7.7353    2.450,   1.4900,        1.2681,  VERATRALDEHYDE:                     33         YES
  26.41      3.8972    2.626,   1.4900,         .6389,  VERATRONE:                          36         YES
  26.71      2.2447    2.656,   2.0500,         .5063,  STEARATE AND OLEATE:                37,38      YES,YES
  27.57      1.9567    2.743,   2.0500,         .4413,  MOSTLY LINOLEATE:                   40         YES
  28.88       .4292    2.875,   1.4900,         .0703,  3,4,5-TMA:                          42         YES
  29.37       .2510    2.925,   1.0000,         .0276,    :                                 --         --
  29.96       .2554    2.984,   2.0500,         .0576,  ARACHIDATE:                         43         YES
  30.47       .4545    3.031,   2.3200,         .1160,  ME RESIN ACID:                      --          NO
  31.01       .5285    3.081,   2.3200,         .1333,  ME RESIN ACID:                      45         YES
  31.73      1.1206    3.148,   2.3200,         .2860,  ME RESIN ACID MW 314:               --          NO
  32.78      7.8243    3.246,   2.3200,        1.9973,  PIMERATE:                           50         YES
  33.60      2.1625    3.322,   2.3200,         .5520,  SANDARACOPIMERATE:                  51         YES
  33.98       .9214    3.357,   2.3200,         .2352,  13-ABIETEN-18-OATE:                 53         YES
  34.54       .6673    3.409,   1.0000,         .0734,    :                                 --         --
  36.23     24.6361    3.566,   2.3200,        6.2888,  ISOPIMERATE:                        55         YES
  38.77       .8480    3.802,   1.0000,         .0933,    :                                 --         --
  41.98     50.9977    4.100,   2.3200,       13.0176,  AB- AND DEHYDROAB-:                 56,57      YES,YES
  42.80       .7900    4.176,   1.0000,         .0869,    :                                 --         --
  44.62      6.8304    4.345,   2.3200,        1.7436,  6,8,11,13-AB, NEOAB-:               58,59      YES,YES
  45.92       .3696    4.465,   2.0500,         .0833,  LIGNOCERATE:                        60          NO
:
                                      TOTAL:    30.7714 MG/L
```

Figure 25 Computer analysis of the chromatogram of Interstate's raw effluent acid and phenol extract

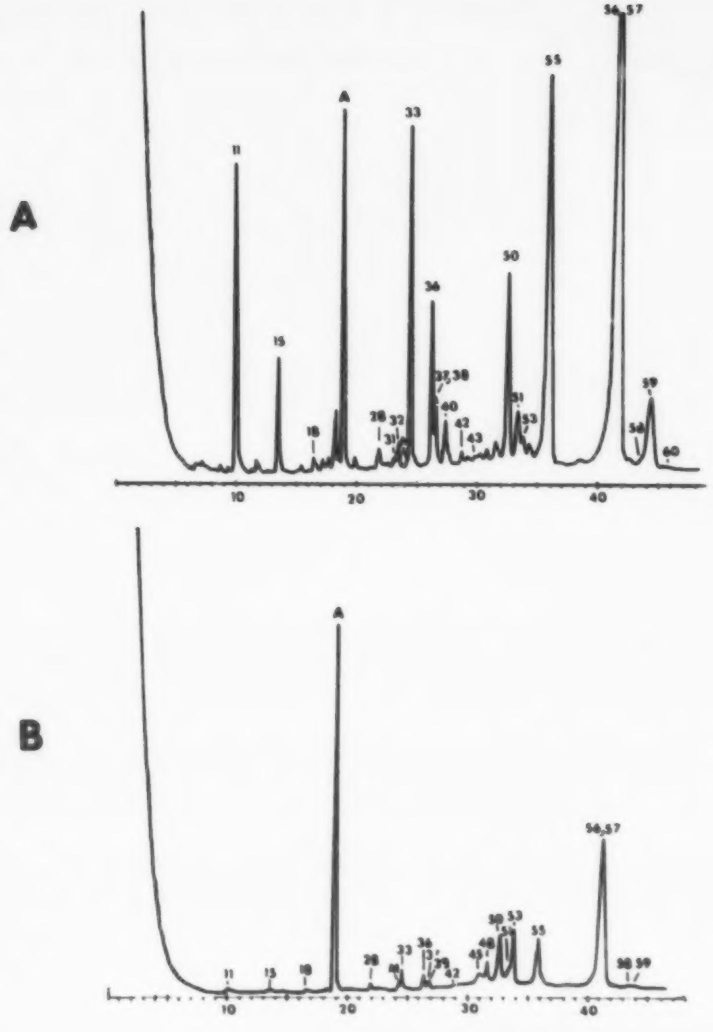


Figure 26 Gas chromatogram of the acid and phenol extract of Interstate's (A) raw effluent and (B) treated effluent

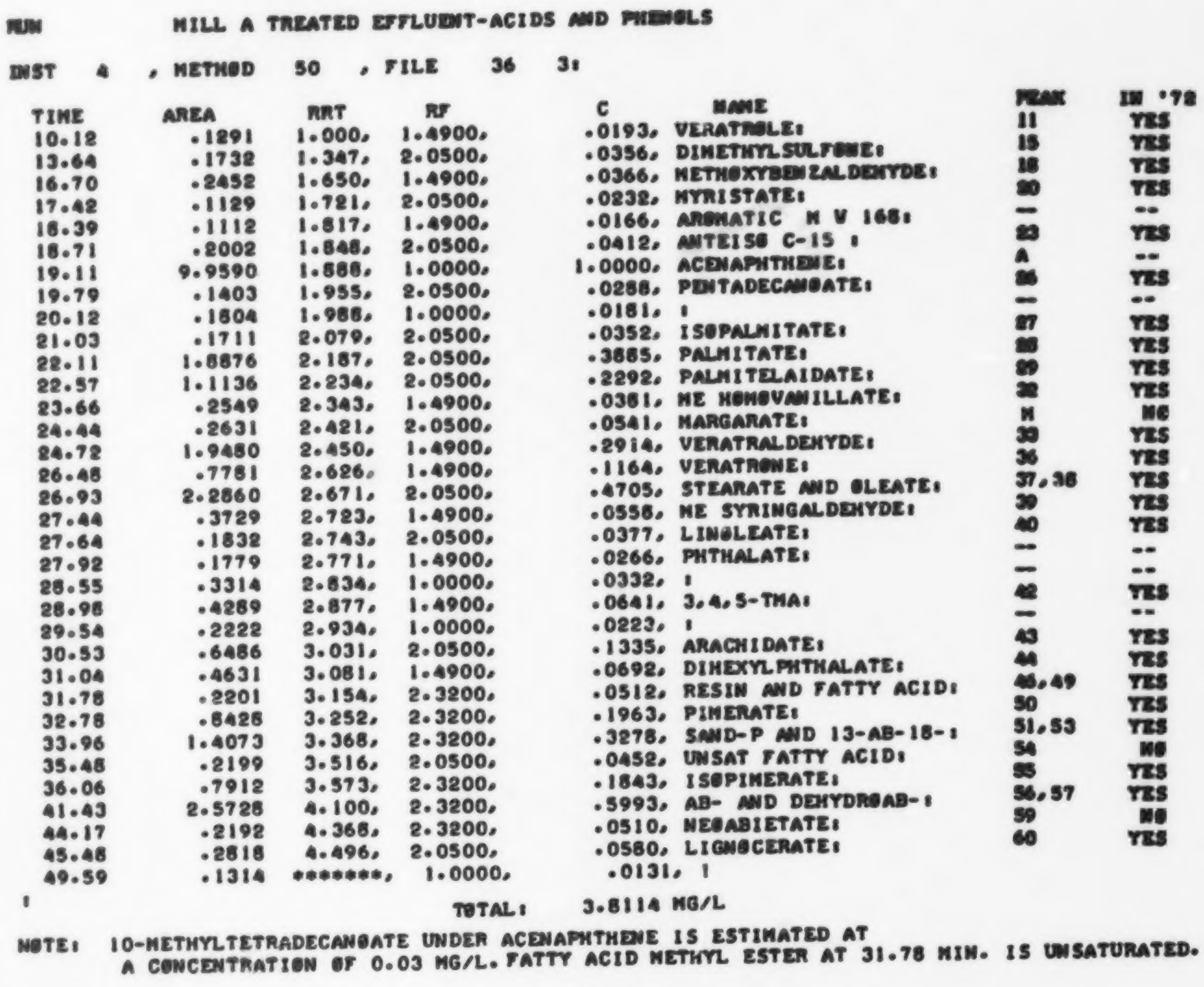
```
RUN         INTERSTATE TREATED EFFL-ACIDS AND PHENØLS

INST   4   , METHØD   50   , FILE    41   3:

  TIME       AREA        RRT      RF            C          NAME                     PEAK     IN '72
  10.12       .1315     1.000,  1.4900,       .0220, VERATRØLE:                     11        YES
                        1.350,  2.0500,             DIMETHYLSULFØNE:                15        YES
  16.65       .1423     1.645,  1.4900,       .0239, METHØXYBENZALDEHYDE:           18        YES
  18.41       .1368     1.819,  1.0000,       .0154, :                              --        --
  19.06      8.8748     1.883,  1.0000,      1.0000, ACENAPHTHENE:                  A         --
  20.07       .1581     1.983,  1.0000,       .0178, :                              --        --
  22.07       .2320     2.186,  2.0500,       .0535, PALMITATE:                     28        YES
  22.74       .2390     2.254,  1.0000,       .0269, :                              --        --
  24.41       .2475     2.423,  2.0500,       .0571, MARGARATE:                     M          NØ
  24.67       .5476     2.450,  1.4900,       .0919, VERATRALDEHYDE:                33        YES
  26.45       .3995     2.630,  1.4900,       .0670, VERATRØNE:                     36        YES
  26.89       .3413     2.675,  2.0500,       .0788, STEARATE AND ØLEATE:           37,38     NØ,YES
                        2.720,  1.4900,             ME SYRINGALDEHYDE:              39        YES
  28.96       .1394     2.885,  1.4900,       .0234, 3,4,5-TMA:                     42        YES
  29.51       .2972     2.941,  1.0000,       .0334, :                              --        --
  30.02       .2021     2.991,  1.0000,       .0227, :                              --        --
  31.06      1.6762     3.093,  2.3200,       .4381, ME RESIN ACID:                 45        YES
  31.72      1.3443     3.157,  2.3200,       .3514, ME RESIN ACID:                 48        YES
  32.74      2.9344     3.257,  2.3200,       .7670, PIMERATE:                      50        YES
  33.91      3.6459     3.371,  2.3200,       .9530, SAND-P AND 13-AB-18-:          51,53     YES,YES
  35.98      2.8126     3.572,  2.3200,       .7352, ISØPIMERATE:                   55        YES
  41.39     11.7420     4.100,  2.3200,      3.0694, AB- AND DEHYDRØAB-:            56,57     NØ,YES
  43.54       .2298     4.309,  2.3200,       .0600, 6,8,11,13-AB, NEØAB-:          58,59     YES,NØ
                        4.460,  2.0500,             LIGNØCERATE:                    60         NØ

                                      TØTAL:    6.9079 MG/L
```

Figure 27   Computer analysis of the chromatogram of Interstate's treated effluent acid and phenol extract

```
RUN          1   MILL A RAW EFFLUENT-ACIDS AND PHENOLS

INST   4   , METHOD   50   , FILE    37    3:

                                                                                      PEAK      IN '78
  TIME        AREA       RRT       RF              C        NAME
  10.14      3.1384    1.000,   1.4900,         .5051,  VERATROLE:                    11        YES
  13.66      1.7837    1.347,   2.0500,         .3817,  DIMETHYLSULFONE:              15        YES
  16.70       .2877    1.646,   1.4900,         .0463,  METHOXYBENZALDEHYDE:          18        YES
  18.48       .4908    1.822,   1.0000,         .0530,  :                             —         --
  19.10      9.2566    1.883,   1.0000,        1.0000,  ACENAPHTHENE:                 A         --
  20.08       .3537    1.980,   1.0000,         .0382,  :                             —         --
  22.10      1.3317    2.184,   2.0500,         .2949,  PALMITATE:                    28        YES
                       2.220,   2.0500,                PALMITELAIDATE:                29        YES
  23.75       .4753    2.350,   1.4900,         .0763,  ME HOMOVANILLATE:             32        YES
  24.73      5.2457    2.450,   1.4900,         .8443,  VERATRALDEHYDE:               33        YES
  26.51      3.0875    2.629,   1.4900,         .4969,  VERATRONE:                    36        YES
  26.82      3.5064    2.661,   2.0500,         .7765,  STEARATE AND OLEATE:          37,38     YES,YES
  27.47       .9110    2.726,   1.4900,         .1466,  ME SYRINGALDEHYDE:            39        YES
  27.66       .7588    2.746,   2.0500,         .1680,  LINOLEATE:                    40        YES
  27.94       .2840    2.774,   1.4900,         .0457,  3,4-DMP:                      41        YES
  28.98       .9289    2.879,   1.4900,         .1495,  3,4,5-TMA:                    42        YES
  30.59       .5789    3.037,   1.0000,         .0618,                                --        --
  31.13       .5133    3.087,   2.3200,         .1286,  ME RESIN ACID:                —         NO
  31.82       .4960    3.152,   2.3200,         .1243,  ME RESIN ACIDS B,C:           46,47     YES,YES
  32.86      4.0798    3.250,   2.3200,        1.0225,  PIMERATE:                     50        YES
  34.10      6.7065    3.366,   2.3200,        1.6808,  SAND-P AND 13-AB-18-:         51,53     YES,NO
  36.20      3.8577    3.563,   2.3200,         .9668,  ISOPIMERATE:                  55        YES
  41.92     26.4614    4.100,   2.3200,        6.6320,  AB- AND DEHYDROAB-:           56,57     YES,YES
  44.11       .3488    4.303,   2.3200,         .0872,  6,8,11,13-AB-:                58        YES
  44.59       .5035    4.350,   2.3200,         .1262,  NEOABIETATE:                  59        YES
  45.81       .4653    4.464,   2.0500,         .1030,  LIGNOCERATE:                  60        NO
 :

                                      TOTAL:    14.9834 MG/L
```

```
RUN          2   MILL A RAW EFFLUENT-ACIDS AND PHENOLS

INST   4   , METHOD   50   , FILE    38    3:

  TIME        AREA       RRT       RF              C        NAME                      PEAK      IN '78
  10.07      3.2236    1.000,   1.4900,         .4950,  VERATROLE:                    11        YES
  13.60      1.8019    1.350,   2.0500,         .3807,  DIMETHYLSULFONE:              15        YES
  16.63       .3241    1.651,   1.4900,         .0497,  METHOXYBENZALDEHYDE:          18        YES
  18.43       .4618    1.830,   1.0000,         .0496,    :                           —         --
  19.05      9.7027    1.891,   1.0000,        1.0000,  ACENAPHTHENE:                 A         --
  20.08       .3204    1.987,   1.0000,         .0330,  :                             —         --
  22.04      1.3318    2.187,   2.0500,         .2812,  PALMITATE:                    28        YES
                       2.220,   2.0500,                PALMITELAIDATE:                29        YES
  23.70       .4879    2.352,   1.4900,         .0749,  ME HOMOVANILLATE:             32        YES
  24.69      5.3897    2.450,   1.4908,         .8276,  VERATRALDEHYDE:               33        YES
  26.46      3.1908    2.625,   1.4900,         .4899,  VERATRONE:                    36        YES
  26.76      3.4873    2.654,   2.0500,         .7368,  STEARATE AND OLEATE:          37,38     YES,YES
  27.42       .9637    2.720,   1.4900,         .1479,  ME SYRINGALDEHYDE:            39        YES
  27.62       .7164    2.739,   2.0500,         .1513,  LINOLEATE:                    40        YES
  27.90       .2862    2.767,   1.4900,         .0439,  3,4-DMP:                      41        YES
  28.93       .9194    2.869,   1.4900,         .1411,  3,4,5-TMA:                    42        YES
  29.50       .3288    2.925,   1.0000,         .0338,  :                             —         --
  30.34       .6329    3.027,   1.0000,         .0652,                                --        --
  31.08       .7586    3.079,   2.3200,         .1814,  ME RESIN ACID:                —         NO
  31.76       .8566    3.145,   2.3200,         .2048,  ME RESIN ACIDS B,C:           46,47     YES,YES
  32.78      4.4606    3.243,   2.3200,        1.0665,  PIMERATE:                     50        YES
  34.00      6.9305    3.361,   2.3200,        1.6571,  SAND-P AND 13-AB-18-:         51,53     YES,NO
  36.08      3.9542    3.563,   2.3200,         .9454,  ISOPIMERATE:                  55        YES
  38.76       .3831    3.822,   1.0000,         .0394,  :                             --        --
  41.63     26.4755    4.100,   2.3200,        6.3304,  AB- AND DEHYDROAB-:           56,57     YES,YES
  44.26       .8086    4.354,   2.3200,         .1933,  6,8,11,13-AB, NEOAB:          58,59     YES,YES
  45.43       .3379    4.467,   2.0500,         .0714,  LIGNOCERATE:                  60        NO
 :

                                      TOTAL:    14.6913 MG/L
```

Figure 28 Computer analysis of the chromatogram of Mill "A's" raw effluent acid and phenol extract (A) run no. 1 and (B) duplicate run



```
RUN         MILL A TREATED EFFLUENT-ACIDS AND PHENOLS

INST   4   , METHOD   50   , FILE    36    3:

  TIME        AREA       RRT       RF             C          NAME                              PEAK       IN '78
  10.12       .1291    1.000,   1.4900,        .0193,  VERATROLE:                          11         YES
  13.64       .1732    1.347,   2.0500,        .0356,  DIMETHYLSULFONE:                    15         YES
  16.70       .2452    1.650,   1.4900,        .0366,  METHOXYBENZALDEHYDE:                18         YES
  17.42       .1129    1.721,   2.0500,        .0232,  MYRISTATE:                          20         YES
  18.39       .1112    1.817,   1.4900,        .0166,  AROMATIC  M W 168:                  —          --
  18.71       .2002    1.848,   2.0500,        .0412,  ANTEISO C-15 :                      23         YES
  19.11      9.9590    1.888,   1.0000,       1.0000,  ACENAPHTHENE:                       A          --
  19.79       .1403    1.955,   2.0500,        .0288,  PENTADECANOATE:                     26         YES
  20.12       .1804    1.988,   1.0000,        .0181,  :                                   —          --
  21.03       .1711    2.079,   2.0500,        .0352,  ISOPALMITATE:                       27         YES
  22.11      1.8876    2.187,   2.0500,        .3885,  PALMITATE:                          28         YES
  22.57      1.1136    2.234,   2.0500,        .2292,  PALMITELAIDATE:                     29         YES
  23.66       .2549    2.343,   1.4900,        .0381,  ME HOMOVANILLATE:                   32         YES
  24.44       .2631    2.421,   2.0500,        .0541,  MARGARATE:                          M          NO
  24.72      1.9480    2.450,   1.4900,        .2914,  VERATRALDEHYDE:                     33         YES
  26.48       .7781    2.626,   1.4900,        .1164,  VERATRONE:                          36         YES
  26.93      2.2860    2.671,   2.0500,        .4705,  STEARATE AND OLEATE:                37,38      YES
  27.44       .3729    2.723,   1.4900,        .0558,  ME SYRINGALDEHYDE:                  39         YES
  27.64       .1832    2.743,   2.0500,        .0377,  LINOLEATE:                          40         YES
  27.92       .1779    2.771,   1.4900,        .0266,  PHTHALATE:                          —          --
  28.55       .3314    2.834,   1.0000,        .0332,  :                                   —          --
  28.98       .4289    2.877,   1.4900,        .0641,  3,4,5-TMA:                          42         YES
  29.54       .2222    2.934,   1.0000,        .0223,  :                                   —          --
  30.53       .6486    3.031,   2.0500,        .1335,  ARACHIDATE:                         43         YES
  31.04       .4631    3.081,   1.4900,        .0692,  DIHEXYLPHTHALATE:                   44         YES
  31.78       .2201    3.154,   2.3200,        .0512,  RESIN AND FATTY ACID:               46,49      YES
  32.78       .8428    3.252,   2.3200,        .1963,  PIMERATE:                           50         YES
  33.96      1.4073    3.368,   2.3200,        .3278,  SAND-P AND 13-AB-18-:               51,53      YES
  35.48       .2199    3.516,   2.0500,        .0452,  UNSAT FATTY ACID:                   54         NO
  36.06       .7912    3.573,   2.3200,        .1843,  ISOPIMERATE:                        55         YES
  41.43      2.5728    4.100,   2.3200,        .5993,  AB- AND DEHYDROAB-:                 56,57      YES
  44.17       .2192    4.368,   2.3200,        .0510,  NEOABIETATE:                        59         NO
  45.48       .2818    4.496,   2.0500,        .0580,  LIGNOCERATE:                        60         YES
  49.59       .1314  *******,   1.0000,         .0131, !
 !
                                        TOTAL:     3.8114 MG/L

NOTE:  10-METHYLTETRADECANOATE UNDER ACENAPHTHENE IS ESTIMATED AT
        A CONCENTRATION OF 0.03 MG/L. FATTY ACID METHYL ESTER AT 31.78 MIN. IS UNSATURATED.
```

Figure 29   Computer analysis of the chromatogram of Mill "A's" outfall acid and phenol extract

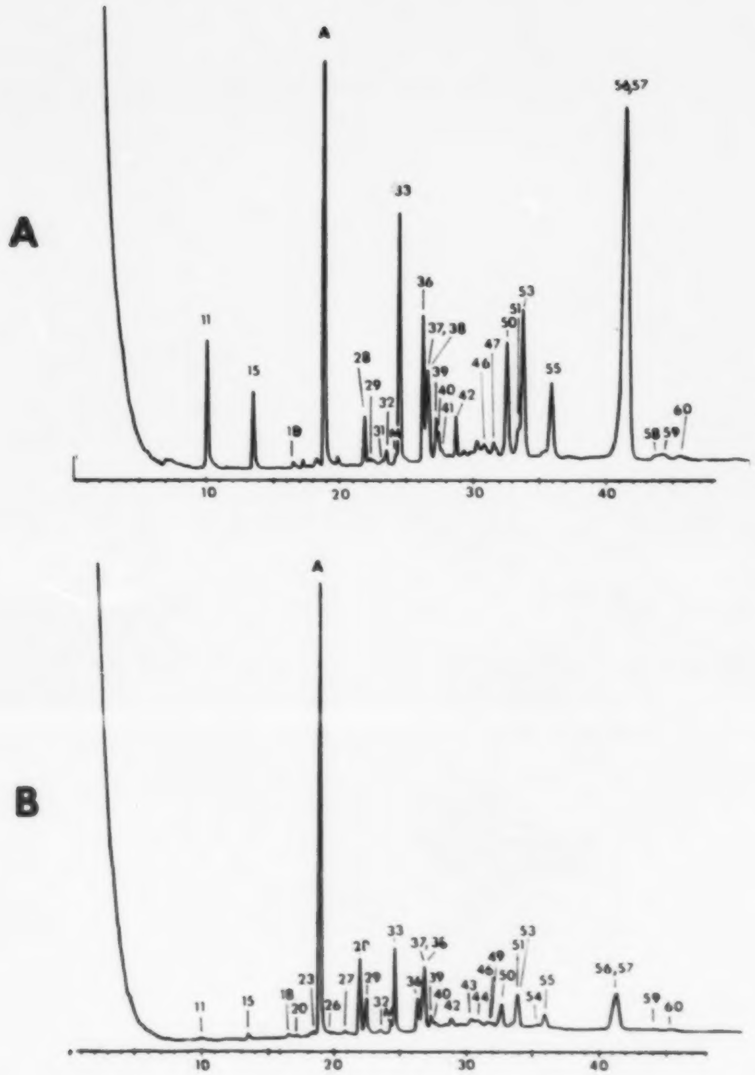


Figure 30   Gas chromatogram of the acid and phenol extract of Mill "A's" (A) raw effluent -- run no. 1 and (B) treated effluent

Comparisons of the 1974 and the 1972 Samples

Table 5 lists TOC, BOD and total volatile components in the acid/phenol extracts from both mills in this study for both sampling times.

Comparison of the collective pollution parameters indicates that BOD removal is around 70-90% in both mill wastewaters after treatment. TOC reduction in the treated wastewaters of both mills probably varies between 60-90%. Because of large amounts of suspended solids in the raw effluent of Mill "A", the 1972 TOC values may be erroneous.

Reduction in total volatile acidic materials is about 65-80% in both mills. The decrease of only 24% in the 1972 sampling of Mill "A" is probably not representative because of faulty sampling, as explained earlier.

If treatment effectiveness is considered with respect to classes of compounds, the phenols appear to be the most susceptable to treatment. Reduction in the volatile phenolic content of each mill was very consistent, ranging in Mill "A" wastewaters from a 73% to a 77% reduction. Volatile phenols were reduced in the Interstate wastewaters by 94% to 95%.

Resin acid reductions in the treated wastewaters ranged from about 50% to 90%. The apparent increase in resin acid content of Mill "A" in 1972 is due to a non-representative grab sample in which the "slug" of effluent being sampled was apparently missed.

Fatty acid content of the Interstate wastewaters was decreased by about 85% to 90%, but there were increases in overall fatty acid concentrations in both the 1972 and the 1974 samples of Mill "A" treated wastewaters. This is probably due to the production of fatty acids by the microbiota in the aerated lagoons. An increase in number of branched and odd-carbon fatty acids was also noted.

To maintain the proper perspective with respect to a mass balance, the total volatile acidic material as well as the three major classes of compounds comprising it is presented as a percentage of the TOC in Table 6. The total of all the volatile acidic components in the wastewater extracts was less than 10% of the total organic carbon content of the wastewaters. The neutral components, discussed in the next section, are usually less than 1% of the TOC. The

Table 5

COLLECTIVE POLLUTION PARAMETER MEASUREMENTS AND TOTAL CONCENTRATIONS OF THE VOLATILE COMPONENTS IN THE ACID-PHENOL EXTRACTS

CONCENTRATIONS (mg/l)

	Mill "A"						Interstate Paper at Riceboro, Georgia					
	1972			1974			1972			1974		
	Raw	Outfall	% Change	Raw	Outfall	% Change	Raw	Outfall	% Change	Raw	Outfall	% Change
BOD_5	323	88	-73	320	45	-86	438	70	-84	440	52	-88
TOC	240	230	- 4	350	350	0	470	200	-57	490	85	-83
Total GC Organics	9.38	7.12	-24	14.98	3.81	-75	21.69	7.24	-67	30.77	6.91	-78
Total Phenols	4.96	1.15	-77	2.31	0.62	-73	5.53	0.27	-95	3.57	0.23	-94
Total Fatty Acids	1.01	1.46	+45	1.36	1.59	+17	1.40	0.14	-90	1.34	0.19	-86
Total Resin Acids	2.04	4.27	+109	10.77	1.39	-87	14.24	6.71	-53	24.37	6.37	-74

*Related to concentrations of total gas chromatographable organic material.

Table 6

VOLATILE ACIDIC COMPONENTS AS PERCENTAGES OF TOC

COMPONENT	PERCENTAGE OF TOC			
	Mill A - 1974		Interstate - 1975	
	Raw Effluent	Outfall	Raw Effluent	Outfall
Total Acidic Volatiles	4.28	1.09	6.28	8.13
Total Phenols	0.66	0.18	0.73	0.27
Total Fatty Acids	0.39	0.45	0.27	0.22
Total Resin Acids	3.08	0.40	4.97	7.49

sum of all the volatile components therefore is still less than 10% of the TOC. Yet in this minority of the mass of dissolved organic material probably lies the bulk of problem-causing compounds--toxic compounds and those causing taste and odor. The dark brown color of kraft pulp mill wastewaters is believed to be due to partially degraded lignin molecules. These high molecular weight non-volatile compounds form a significant portion of the balance of the TOC. Other contributors to the TOC are the carbohydrates and, to a lesser extent, tannins and various other highly polar or non-volatile compounds.

The increase in the proportion of the TOC represented by total volatiles and resin acids in the Interstate wastewaters is a reflection of the greater decrease in the non-volatile portion of the organic content as compared to the volatile and resin acid content. Lime flocculation probably removed a large amount of the non-volatile organics.

The major difference in the volatile organic content of wastewaters from the two mills was the fatty acid content. Whereas the fatty acids decreased significantly during the Interstate treatment, they increased during treatment in Mill "A".

Reduction in BOD, TOC, total GC peak areas, and phenolic and resin acid content were similar in the wastewaters from the two mills.

SECTION VI

IDENTIFICATION OF TERPENES

The neutral volatile fraction of the paper mill wastewater extracts was not subjected to as complete a characterization as was the acidic and phenolic fraction. There were several reasons for this decision:

- Dr. Bjorn Hrutfiord, University of Washington, has been conducting a similar study on an EPA grant for the past 2 years and has concentrated primarily on the terpene fraction and on a sugar fraction.[42]

- Our results indicated that the terpenes and other neutral volatiles are about one-tenth the concentration of the acids and phenols in the raw wastewaters.

- The terpenes appear to be generally susceptible to biological treatment and are minor constituents in the treated wastewaters being discharged.

GC-MS ANALYSIS

Chemical profiles of the neutral volatiles from the extracts of both mills are shown in Figures 31 and 32. Table 7 lists the individual compounds corresponding to the peak numbers in Figures 31 and 32. Since these samples were taken in 1972, they may not be quantitatively representative of a slug of the effluent. The concentrations of compounds in each sample are probably slightly low because extraction efficiencies are never 100%. Several compounds were detected and identified even though their concentrations were less than 1 part per billion.

Gas chromatograms from the 1974 sampling of the raw effluents and the treated effluents of both mills are shown in Figures 33 and 34. No attempt was made to identify these compounds because of lack of time. However, most of the GC peaks have similar relative retention time and areas as those from the 1972 neutral volatile extracts.

GC-IR ANALYSIS

Two of the 1972 neutral volatile extracts from Mill A were analyzed by a Digilab Fourier Transform infrared spectro-

Table 7. NEUTRAL VOLATILES IDENTIFIED IN BOTH KRAFT PAPER MILL EFFLUENTS WITH APPROXIMATE CONCENTRATIONS

Peak No.	Compound Identified	Confirmed By	Approximate Conc. in mg/l Mill "A" Sample Points 1	2	3	4	Approximate Conc. in mg/l Interstate Sample Points 1	2	3	4
4	Methyl trisulfide		0.003	0.004	0.008	0.001	--	--	--	--
62	Fenchone	MS,GC	0.007	0.007	0.015	0.015	0.015	0.002	0.001	<0.001
63	Hexachloroethane		--	--	--	--	--	--	--	<0.001
64	Sabinene		--	--	--	--	--	--	--	0.003
65	Unidentified terpene ketone		0.055	0.050	0.055	0.045	0.040	0.010	0.015	0.004
66	Camphor	MS,GC,IR	0.045	0.045	0.060	0.090	0.090	0.015	0.020	0.035
67	Unidentified terpene ketone		0.045	0.040	0.050	0.045	0.045	0.006	0.020	0.008
68	Unidentified terpene ketone		0.020	0.020	0.025	0.020	0.020	0.003	0.008	0.002
69	Fechyl alcohol	GC	0.065	0.065	0.035	0.010	0.105	0.040	0.060	--
70	Unidentified terpene ketone		0.025	0.025	0.020	0.004	0.015	0.005	0.009	--
71	Terpene-4-ol	MS,GC	0.050	0.045	0.040	0.010	0.030	0.010	0.015	<0.001
72	2-Formylthiophene		0.010	--	--	--	--	--	--	--
73	Methyl chavicol	MS,GC	0.045	0.040	0.030	--	0.030	0.010	0.020	--
9	Borneol	MS,GC,IR	0.275	0.200	0.155	0.090	0.470	0.200	0.260	--
10	α-Terpineol	MS,GC	0.645	0.700	0.625	--	0.490	0.215	0.280	0.080
11	Veratrole	MS,GC	0.020	0.015	0.015	0.008	0.015	0.004	0.008	--
74	2-Acetylthiophene	GC,IR	0.025	0.025	0.030	0.025	0.012	0.002	0.004	<0.001
75	Myrtenol		0.010	0.010	0.008	--	0.008	--	--	--
76	2-Propionylthiophene	GC,IR	0.025	0.025	0.025	0.010	0.020	0.005	0.010	<0.001
77	Anethole		0.007	--	--	--	--	--	--	--
78	Benzyl alcohol		0.013	0.012	0.008	--	0.025	0.004	0.007	--
79	Methyl eugenol	GC	0.002	0.001	--	--	--	--	--	--
80	Unidentified terpene alcohol		0.006	0.007	0.010	--	0.030	0.015	0.015	0.008
81	Unidentified aromatic similar to methyl isoeugenol (MW=178)		0.006	0.008	0.009	--	0.009	0.006	0.008	--
30	Ethyl palmitate	MS,GC	0.006	--	--	--	--	--	--	--
82	Unidentified monounsaturated C_{19} fatty acid methyl ester		0.038	--	--	--	--	--	--	--
83	Unidentified diunsaturated C_{19} fatty acid methyl ester		0.016	--	--	--	--	--	--	--
84	Unidentified phthalate diester		--	--	--	--	--	--	--	--
Total			1.464	1.344	1.223	0.711	1.469	0.552	0.760	0.145

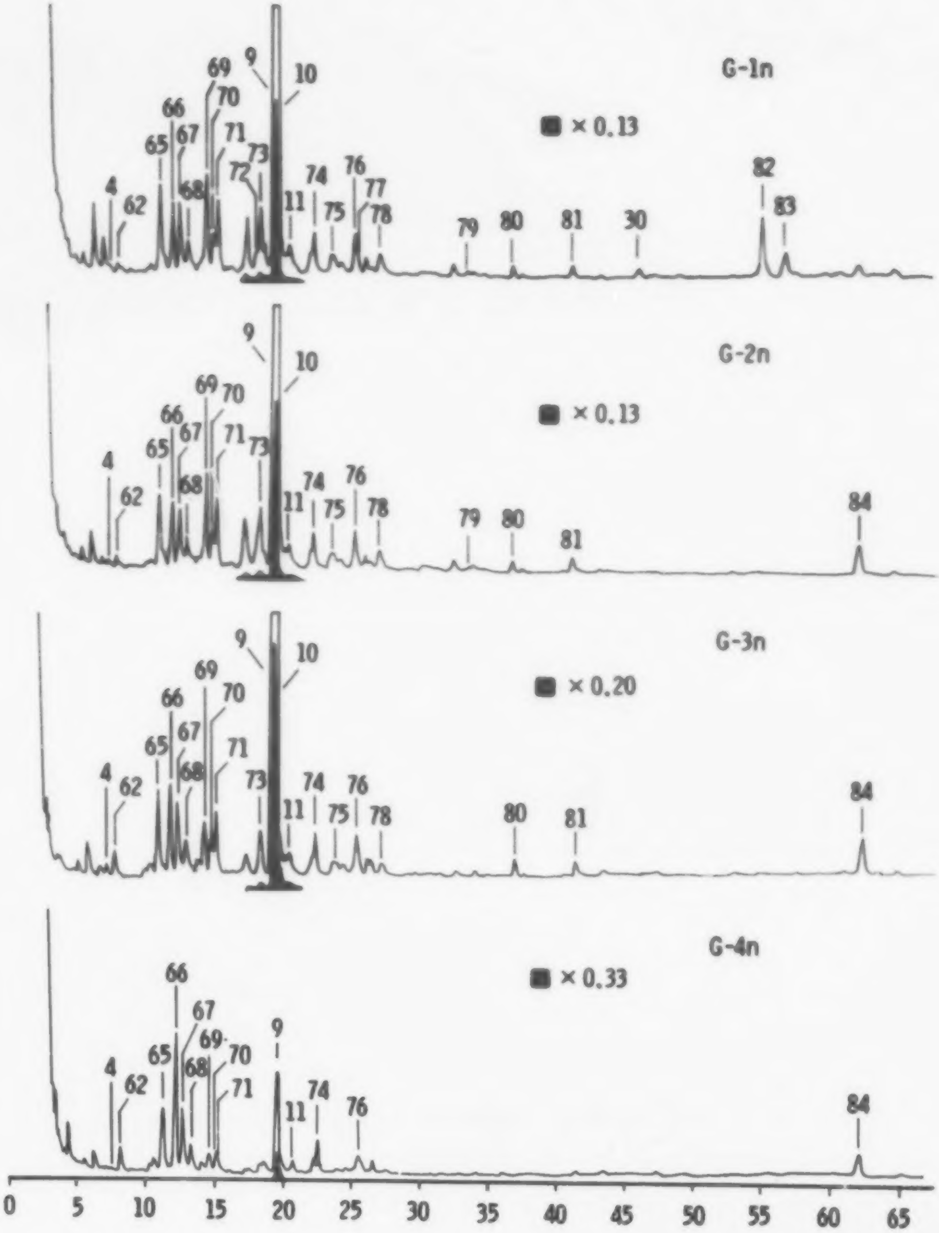


Figure 31 Chemical profile of neutral volatiles from Mill "A"; sample points 1-4

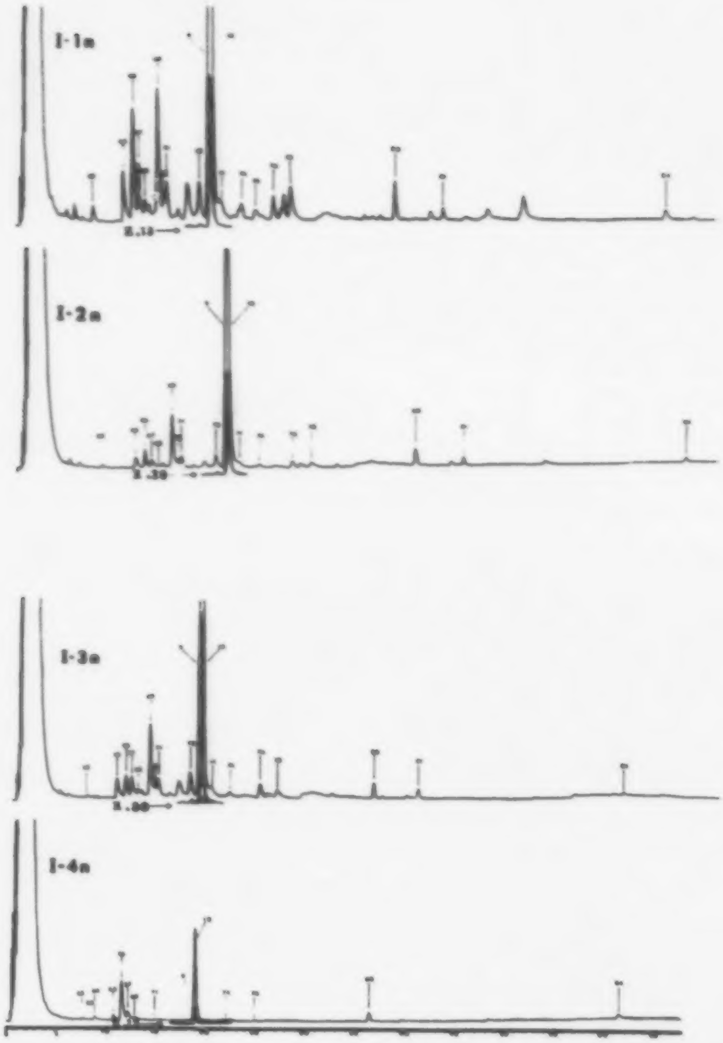


Figure 32   Chemical profile of neutral volatiles from the Interstate mill at Riceboro; sample points 1-4

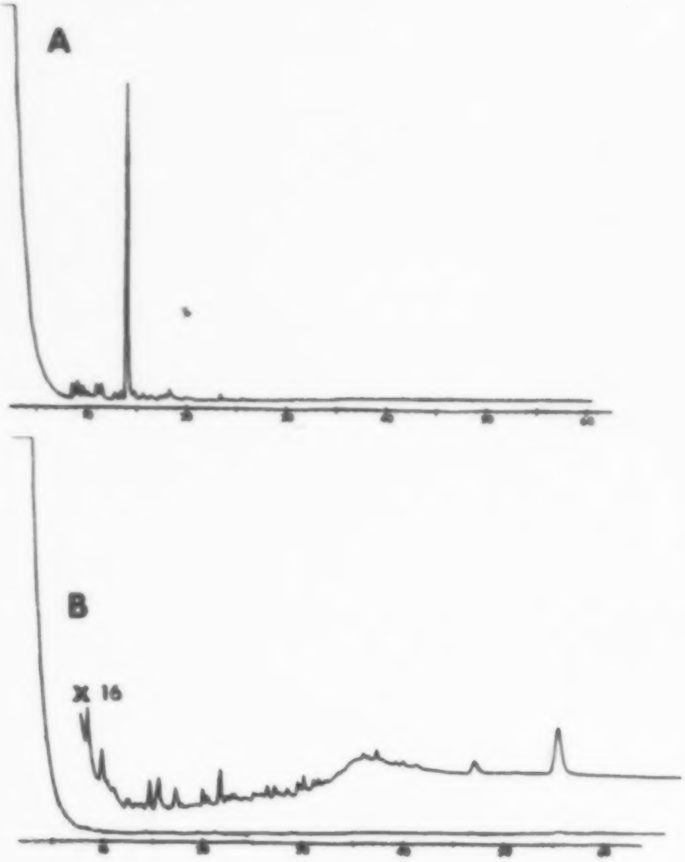


Figure 33 Gas chromatogram of neutral volatile extract from Mill "A's" raw effluent (A) and (B) treated effluent

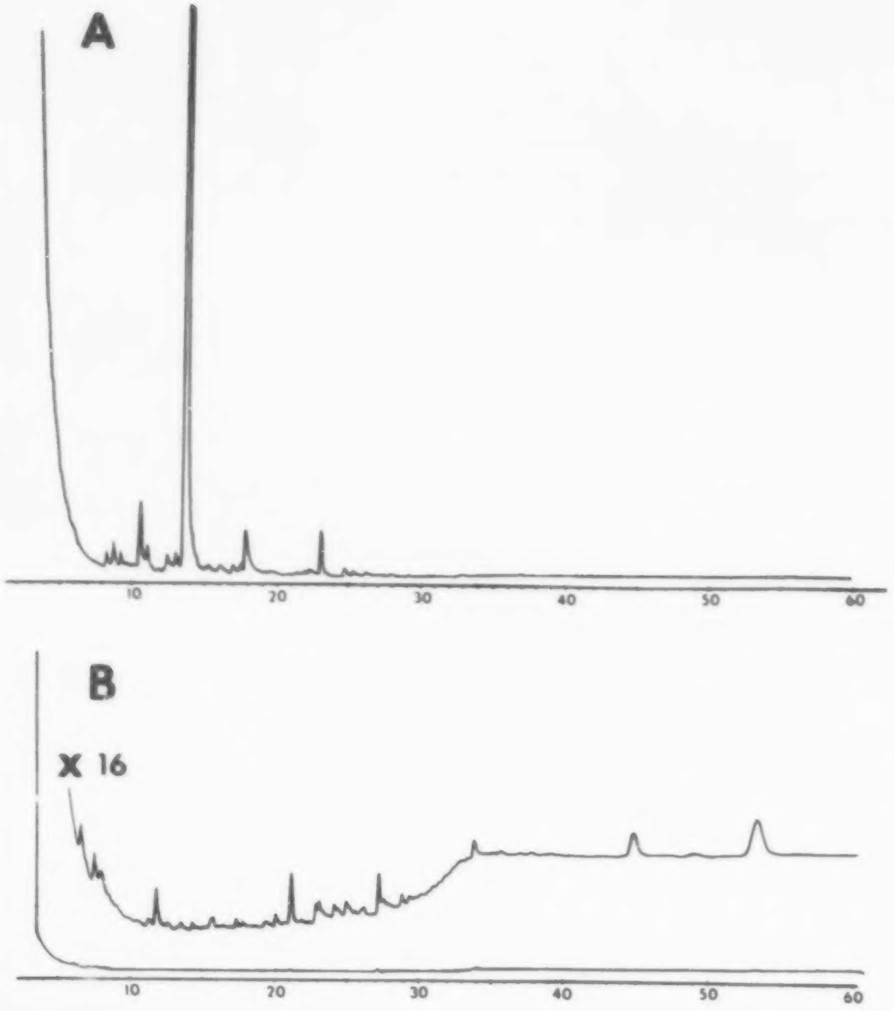


Figure 34 Gas chromatogram of neutral volatile extract from Interstate's raw effluent (A) and (B) treated effluent

photometer interfaced with a gas chromatograph (GC-IR). Spectra of the eluting compounds are obtained "on the fly" as they are with the GC-MS system.

The gas chromatograms of the two samples analyzed by GC-IR are shown in Figure 35. GKR-3 and GKR-4 correspond to G-3n and G-4n, respectively, in Figure 31. A different peak notation system was used in Figures 35-45. The peak numbers in these figures correspond to the identifications in Table 6 in the following way:

GC-IR Designation	Table 6 Designation	Compound Name
13	67	unidentified ketone
14	68	unidentified ketone
15	69-71	3 compounds
16	-	-
17	73	methyl chavicol
18	9,10	borneol and α-terpineol
19	74	2-acetylthiophene
20	75	myrtenol
21	-	-
22	76	2-propionylthiophene
23	-	-
24	78	benzyl alcohol
a	65	unidentified ketone
b	66	camphor
c	-	-
d	67	unidentified ketone
e	68	unidentified ketone

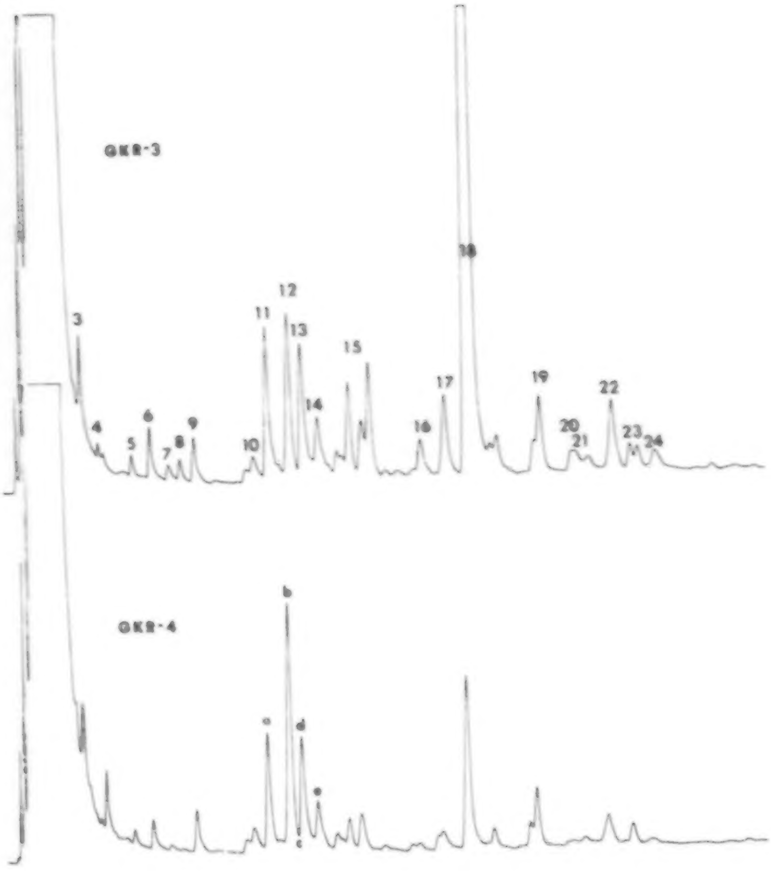


Figure 35 Gas chromatograms of Mill "A" neutral volatile extracts analyzed by GC-IR; sample points 3-4

The GC-IR spectra of the peaks in Figure 35 are shown in Figures 36-41.

Although the exact structures of peaks 11,13, and 14(a,d, and e in GKR-4) were not identified from the GC-IR spectra (Figs. 38 and 41), they were all ketones. Computer matching of the mass spectra of these same peaks had led to tentative identifications of 1-cyclohexenyl methyl ketone, 4-nonyne, and 3-cyclohexen-1-yl methyl ketone, respectively. The presence of ketone carbonyl absorption in the infrared spectra eliminates the possibility that one is 4-nonyne and confirms the methyl alkyl ketone structures of all three compounds.

Four of the GC-IR identifications were confirmed by comparing the sample spectra with standards. The spectra of camphor, borneol, 2-acetylthiophene, and 2-propionylthiophene are shown in Figures 42-45 respectively with the corresponding sample spectra. The two terpenes had previously been confirmed by GC-MS but the two thiophene isomers were confirmed only by GC-IR.

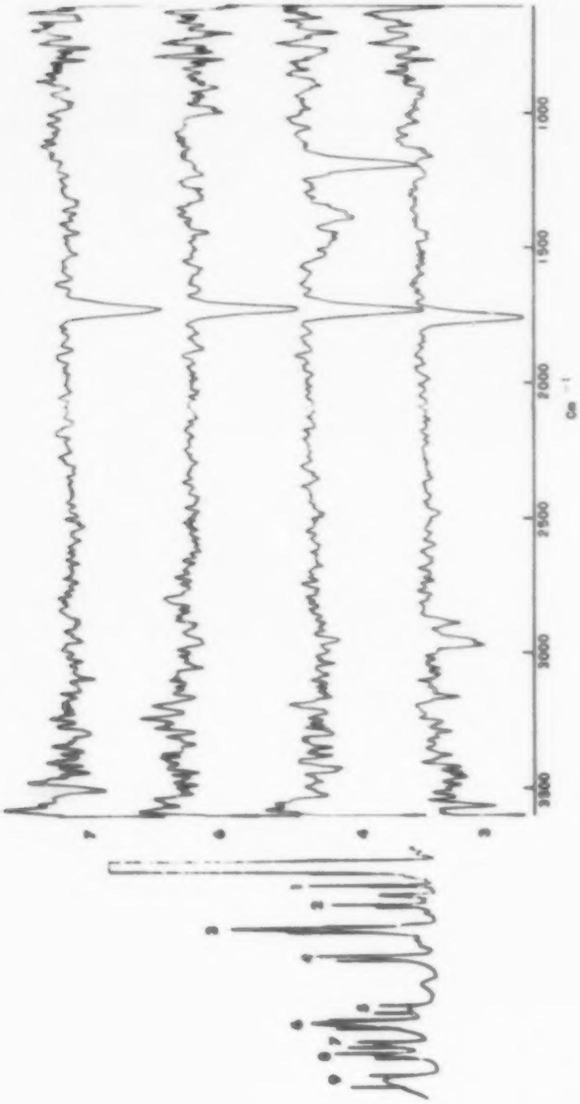


Figure 36 GC-IR spectra of peaks 3-7; sample point 3 with inset of the corresponding portion of the gas chromatogram

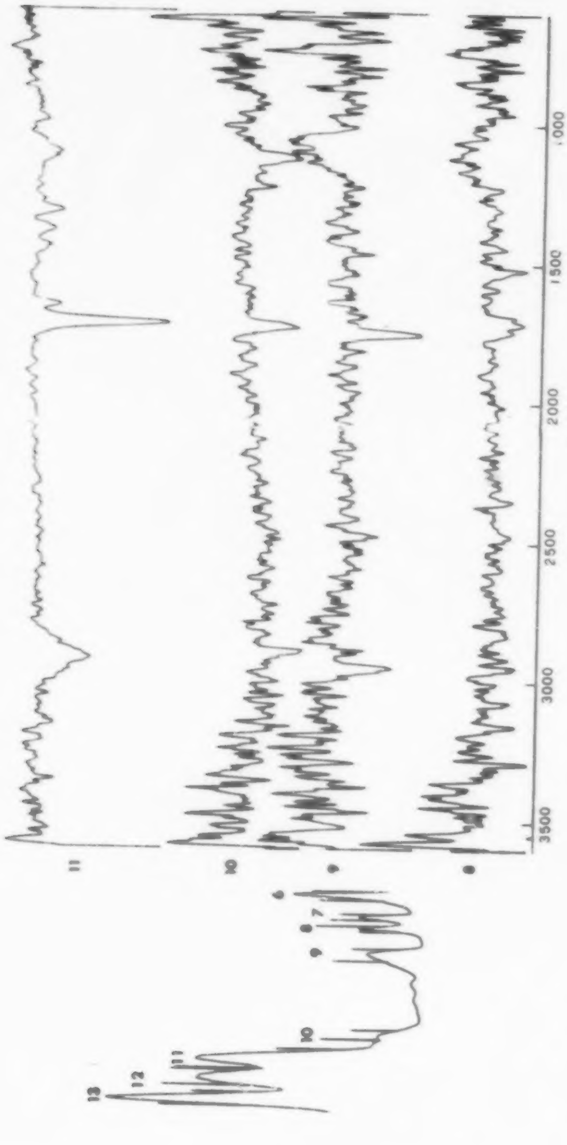


Figure 37 GC-IR spectra of peaks 8-11; sample point 3 with inset of the corresponding portion of the gas chromatogram

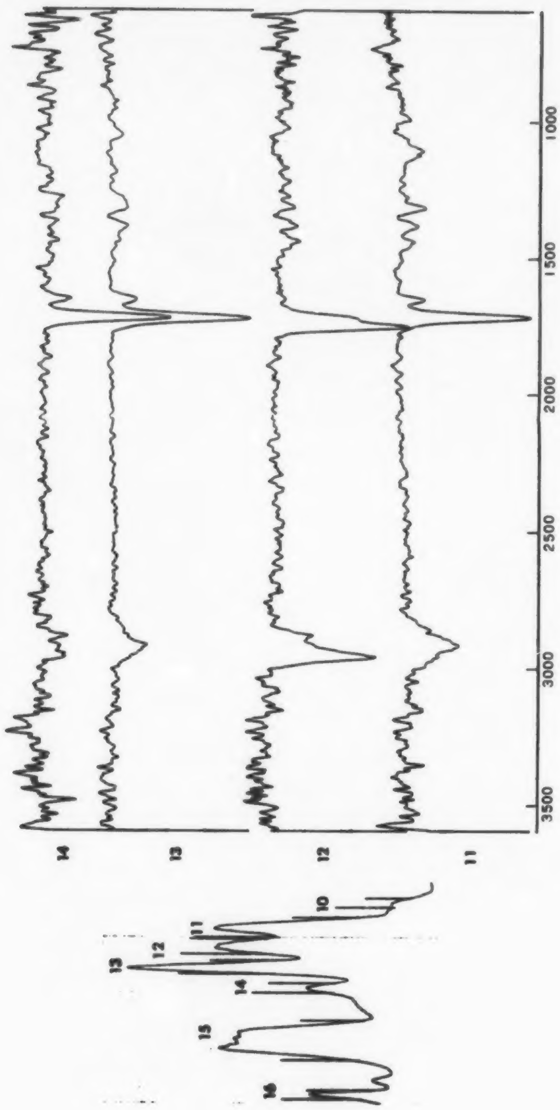


Figure 38 GC-IR spectra of peaks 11-14; sample point 3 with inset of the corresponding portion of the gas chromatogram

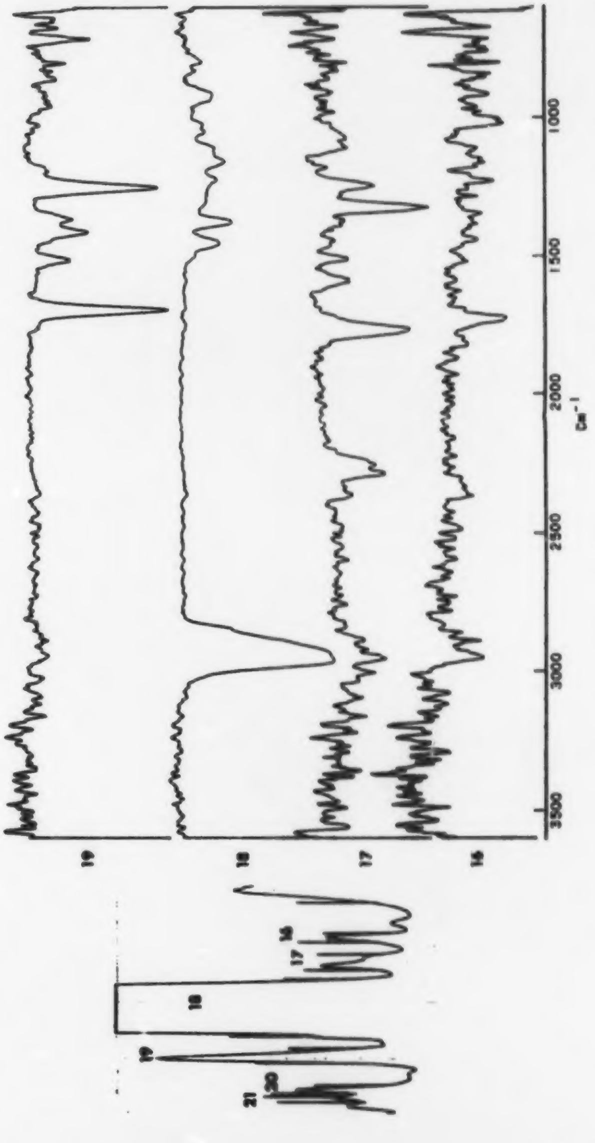


Figure 39   GC-IR spectra of peaks 16-19; sample point 3 with inset of the corresponding portion of the gas chromatogram

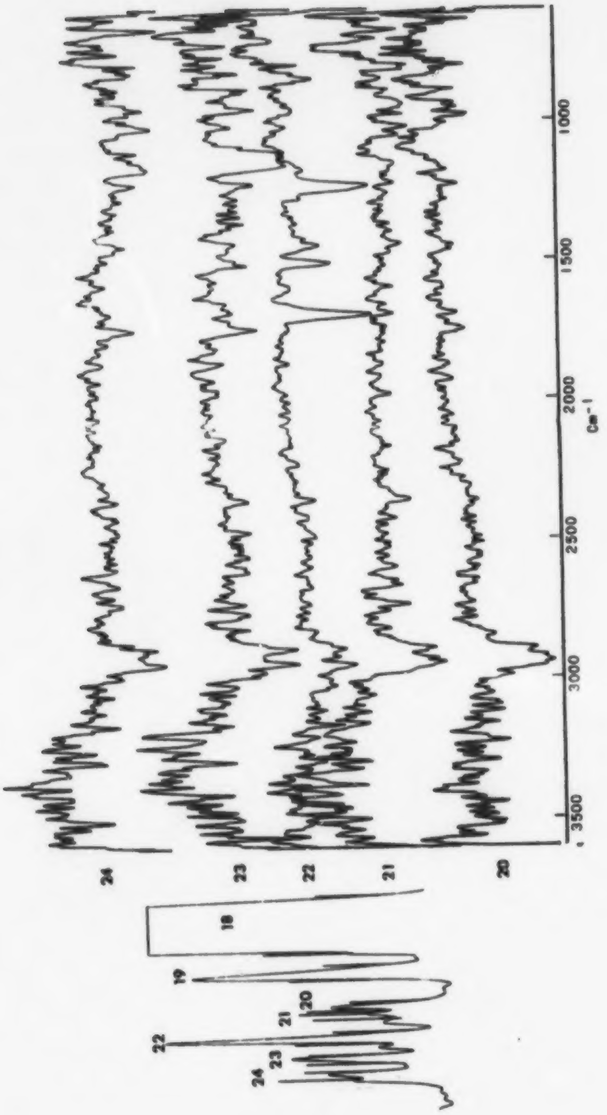


Figure 40 GC-IR spectra of peaks 20-24; sample point 3 with inset of the corresponding portion of the gas chromatogram

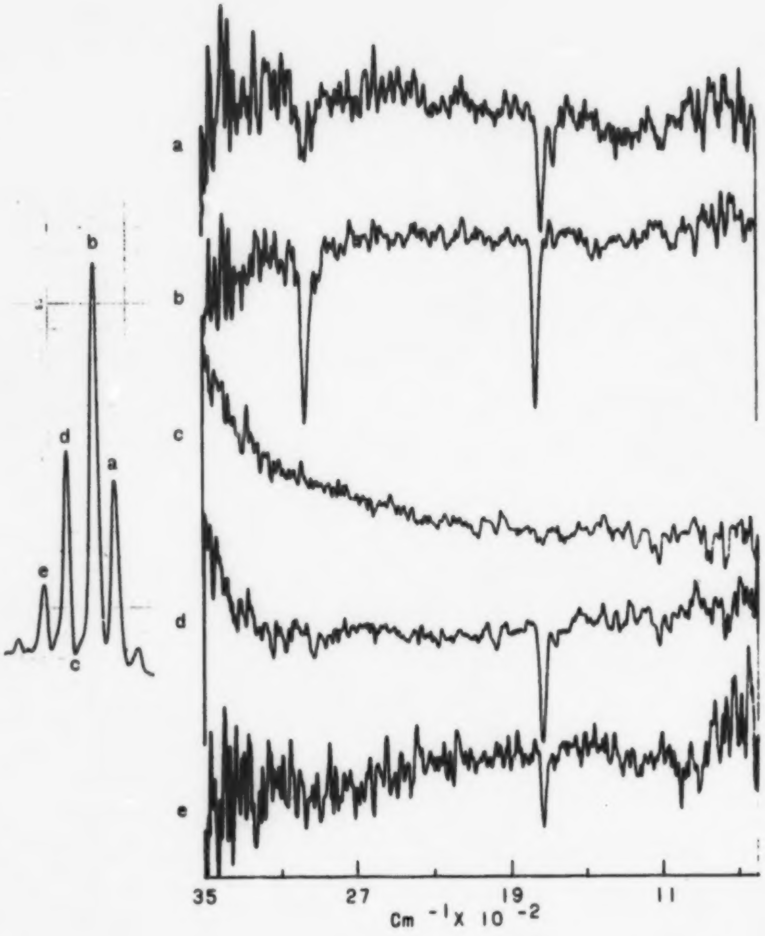


Figure 41 GC-IR spectra of peaks a-e; sample point 4 with inset of the corresponding portion of the gas chromatogram

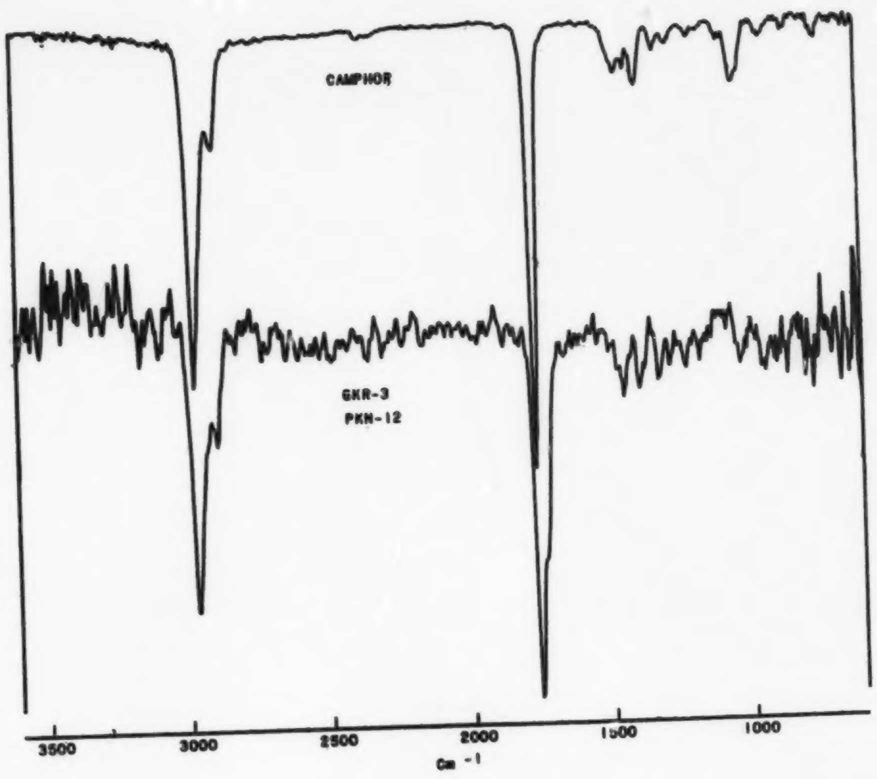


Figure 42 GC-IR spectrum of camphor and corresponding GC-IR spectrum from sample peak

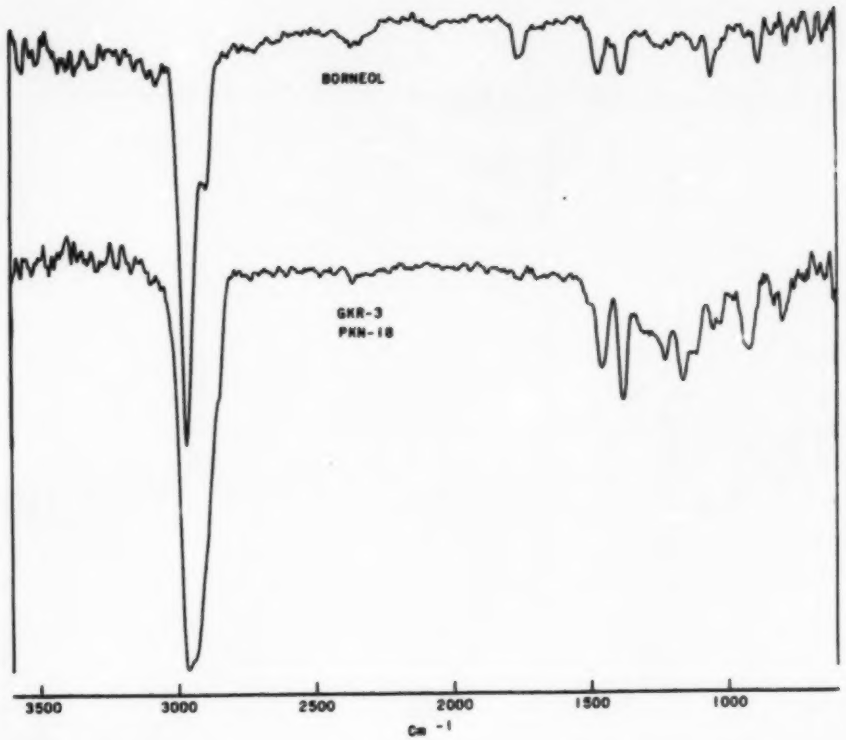


Figure 43 GC-IR spectrum of borneol and corresponding GC-IR spectrum from sample peak

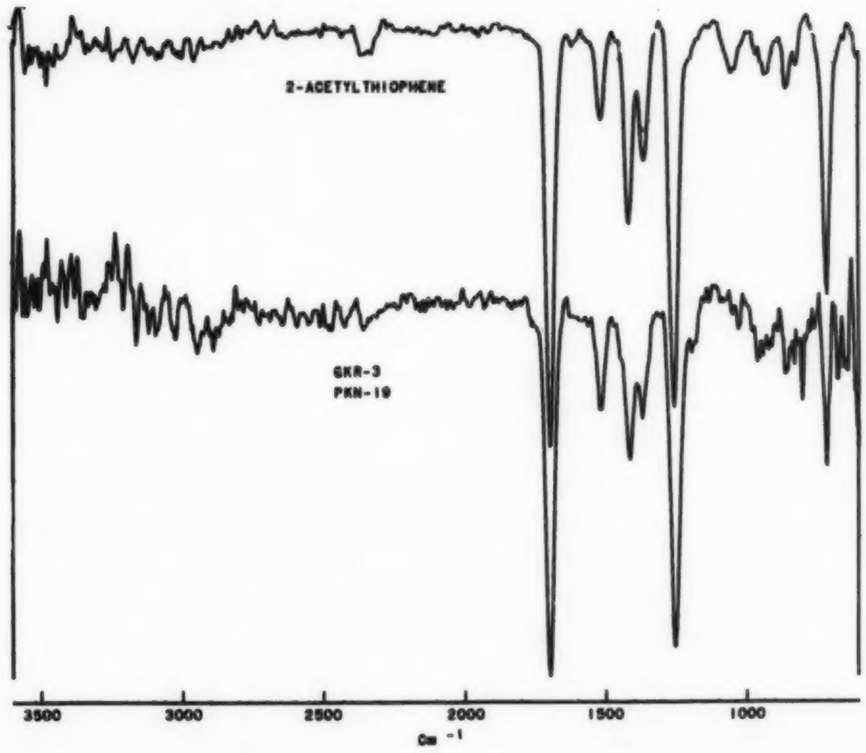


Figure 44 GC-IR spectrum of 2-acetylthiophene and corresponding GC-IR spectrum from sample peak

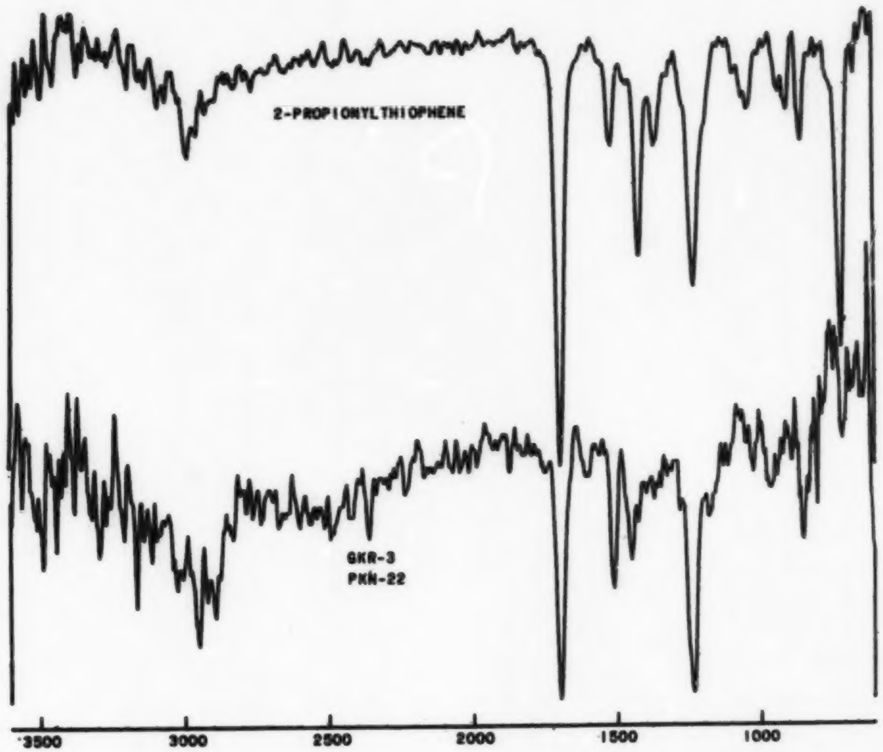


Figure 45 GC-IR spectrum of 2-propionylthiophene and corresponding GC-IR spectrum from sample peak

SECTION VII

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SECTION VIII

APPENDICES

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A. Procedure for diazomethane methylation 98

B. Procedure for dimethyl sulfate methylation 99

APPENDIX A

Procedure for diazomethane methylation

The apparatus is shown in Figure 6.

1. Evaporate the sample extract just to dryness with a stream of nitrogen in a centrifuge tube, the bottom tube of a Kuderna-Danish apparatus, or the sample storage vial. A small amount of methylene chloride may be retained, but the presence of chloroform may produce artifacts. Dissolve the extract in 0.5-1.0 ml distilled-in-glass ethyl ether.

2. Add about 5 ml of distilled-in-glass ether to the first tube of the apparatus to saturate the nitrogen carrier gas with ether. Add 0.7 ml of carbitol [2-(2-ethoxyethoxy)ethanol], 1.0 ml of 37% aqueous KOH (not over 2 days old), and 0.1-0.2 g of N-methyl-N-nitroso-p-toluenesulfonamide ("Diazald," Aldrich Chemical Co.) to the second tube. The base immediately begins to release diazomethane from the sulfonamide.

3. Immediately position the second test tube and adjust the nitrogen flow to about 10 ml per minute. Caution: Diazomethane is an extremely toxic and explosive gas. A good fume hood and safety glasses are mandatory. No chipped glassware should be used, as rough glass surfaces catalyze decomposition of diazomethane.

4. Position the third tube (a safety trap to prevent reagent carry-over) and the sample tube to bubble the nitrogen and diazomethane gas mixture through the sample. Continue the reaction until the slight yellow color of diazomethane persists in the sample solution (from a few seconds to 30 minutes, depending upon the sample concentration). In the case of dark colored extracts in which the diazomethane is not visible, a reaction time of 30 minutes is recommended.

5. Allow the esterified sample to stand unstoppered in the hood for 15 to 30 minutes to allow excess diazomethane to escape from the ether solution. Discard all waste from the reaction with care and rinse the apparatus with acetone. Evaporate the sample to the volume necessary for gas chromatography.

APPENDIX B

Procedure for dimethyl sulfate methylation

The apparatus is shown in Figure 7.

1. Bring the original sample (300 ml) to pH 11 with NaOH and extract with chloroform to remove neutral and basic compounds.

2. A 500-ml 3-neck (standard taper 24/40) round bottom flask, equipped with a fourth neck for a thermometer, is fitted with two pressure-equalizing addition funnels, the probe of a single-probe pH meter, and a magnetic stirrer.

3. Nitrogen is introduced into the top of the first addition funnel and exits from the top of the second one. Place forty ml of Eastman reagent grade dimethyl sulfate into the first addition funnel and a 50% solution of sodium hydroxide (80 ml) into the second.

4. Pour the sample into the flask and flush the system with nitrogen.

5. After raising the temperature to 85° C, begin dropwise addition of both the dimethylsulfate and the sodium hydroxide solution. Maintain temperature between 80 and 90° C. (Caution--exothermic reaction. Have ice available to add to water bath.) and the pH between 10.5 and 11. Since dimethylsulfate is not readily soluble in water vigorous stirring must be used. The addition time is about 1 hour.

6. After all the dimethylsulfate is added, maintain the reaction vessel at 85-90° C for an additional 15-20 minutes and then cool to room temperature.

7. Add 5 ml concentrated ammonium hydroxide to destroy excess dimethylsulfate and re-extract the reaction mixture with chloroform to remove the methyl esters of acids and the methyl ethers of phenols.

8. Dry the chloroform extract and evaporate to the appropriate volume for GC analysis.